BIOBEDS: A NEW APPROACH TO PESTICIDE RINSATE DISPOSAL

A Thesis Submitted to the College of Graduate Studies and Research
In Partial Fulfillment of the Requirement for
The Degree of Master of Science
In the Department of Soil Science
University of Saskatchewan
Saskatoon, Canada

By
Dean Lucas Lyonga Ngombe

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirement for a postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying this thesis in any matter, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis or in their absence, by the Head of the Department or Dean of the college in which my thesis work was done. It is understood that any copying, publication, or use of this thesis or parts thereof for financial gain shall not be allowed without approval by the University of Saskatchewan and my written permission. It is also understood that due recognition will be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Request for permission to copy or to make other use of material in this thesis in whole or in part should be addressed to:

Head of the Department of Soil Science
University of Saskatchewan
51 Campus Drive
Saskatoon, Saskatchewan, Canada
S7N 5A8.

ABSTRACT

Modern agriculture relies heavily on the use of chemicals for pest control to increase crop yields. However, improper handling and disposal of pesticides and pesticide waste may compromise surface water and groundwater quality. In farmyards, point-source contamination includes spills occurring during filling operations, sprayer rinsate, leaks, internal and external sprayer cleaning, and spray leftovers, and accounts for 40 to 90% of surface water and groundwater contamination by pesticides. Over the years, various biopurification systems have been developed to minimize the risk of point-source contamination by pesticides. One such system is the biobed. It consists of an active matrix (straw, peat or compost, and topsoil) in a ratio of 2:1:1 (v/v/v) in a pit. The effectiveness of the biobed mix is based on its ability to adsorb pesticides into its active matrix or stimulate their rapid biodegradation by offering suitable biological and physicochemical conditions for optimum microbial activity. The objective of this project was to study the efficiency of biobeds under Saskatchewan climatic conditions.

Two laboratory studies and one field study were conducted in Saskatoon, SK. In the first laboratory study, the degradation of 2,4-D dimethylamine salt (2,4-D DMA) in single and multiple additions to the biobed mix and topsoil was investigated at 20 and 15 ^{o}C, respectively. In both studies, net CO_2 evolution was correlated with 2,4-D degradation. Degradation of 2,4-D was more rapid in the biobed mix, with more than 99.9% degradation in 10 d, compared to topsoil with only 35% degradation in 28 d of incubation at 20 ^{o}C. In the multiple additions experiment, more than 99.9 and 70% of the applied amount of 2,4-D DMA was degraded in the biobed mix and topsoil, respectively, within 60 d of incubation at 15 ^{o}C. A mass balance showed that 93 and 51% of the C added as 2,4-D dimethylamine salt was mineralized within 60 d of incubation at 15 ^{o}C in the biobed mix and topsoil, respectively. These results suggest that net CO_2 could be used as an indicator of 2,4-D DMA degradation in the biobed mix and topsoil used.

A second laboratory experiment examined the degradation of seven pesticides at three temperatures (5, 13, and 20 ^{o}C). Pesticide degradation was more rapid at 20 ^{o}C compared to 13 and 5 ^{o}C. A significant interaction (sampling time x temperature) was observed in the degradation of thifensulfuron-methyl, 2,4-D DMA, pyrasulfotole, and bromoxynil. For metsulfuron-methyl, tribenuron-methyl, and thiencarbazone-methyl,

degradation was a function of sampling time and temperature with no interaction. After 35 d of incubation at 20 ^{o}C, 38, 94, 99, 77, 77, and 99% of applied amounts of metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and bromoxynil, respectively, were degraded while more than 99% of the applied amount of 2,4-D DMA was degraded within 7 d. The results suggest that high pesticide degradation could be anticipated during warm conditions (summer) compared to fall or spring.

A field study examined the degradation of six pesticides in two designs of biobed (the traditional below-ground biobed and a newly designed above-ground biobed) during two growing seasons. The above-ground biobed reached peak temperatures faster than the traditional below-ground biobed and was more efficient in water management. The above-ground biobed was more vulnerable to pesticide leaching out of the bottom compared to the below-ground biobed. The most leached pesticide was metsulfuron-methyl, with 4 and less than 0.01% of the applied amount leaching out of the bottom of the above-ground biobed and below-ground biobed, respectively. More than 99% of the applied amounts of tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D DMA were either retained by the biobed matrix or degraded within 2 growing seasons in both biobeds.

This research shows that biobeds are capable of degrading herbicides from different chemical classes and could be used to reduce surface water and groundwater contamination arising from point sources in Saskatchewan in particular and the prairies in general. However, consideration in their design should include leaching potential, water management, early-season biobed temperature and they must be closed.

LIST OF ABBREVIATIONS

a.i.	Active ingredient
AAFC	Agriculture and Agri-Food Canada
AGB	Above-ground biobed
BGB	Below-ground biobed
FOK	First order kinetics
GUS	Groundwater ubiquity score
MB	Microbial biomass
MB-C	Microbial biomass carbon
MB-N	Microbial biomass nitrogen
ODW	Oven-dry weight
OM	Organic matter
SOC	Soil organic carbon
TOC	Total organic carbon
WHC	Water holding Capacity
WRF	White rot fungi

ACKNOWLEDGEMENTS

First and foremost, I would like to extend my sincere appreciation to my wife (Esther Ndatake Efeme) who encouraged me to leave Montreal and pursue a graduate degree at the U of S, to my sisters-in-law Namondo Efeme and Maondo Efeme for taking good care of my wife and two sons (Sammy and Dean Jr.). Without your love, encouragement, and support, this work would not have been possible.

I am very grateful for the support and guidance I received from my supervisors, Drs. J. Diane Knight and Thomas Martin Wolf, especially their feedbacks, patience and encouragement during the writing process and the opportunities they offered to me to present my work at the national and international stage. The guidance and suggestions I received from my supervisory committee, Drs. Bing Si, Richard Farrell, and Allan Cessna and my external examiner Dr. Charles Maulé contributed greatly to this work. I would like to thank Brian Caldwell for his invaluable support both in the lab and field, Jonathan Geissler for his assistance in the lab, Jonathan Bailey for performing all the chemical analyses and Murray Belyk from Bayer CropScience for facilitating this project.

During my stay at the Department of Soil Science, I came across nice people. I appreciate all your love and support during good and bad times. Your positive attitude gave me encouragement. Thumbs up to the staff in the Knight/Farrell lab, all graduate students, staff, and personnel at the Department of Soil Science.

I am grateful to Bayer CropScience, Agriculture and Agri-Food Canada and Saskatchewan Ministry of Agriculture – Strategic Research Program for funding this research.

DEDICATION

To my lovely and handsome sons, Sammy Eyole Lyonga Ngombe and Prince Dean Jr. Masoma Lyonga Ngombe.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

In recent years, there has been growing concern that improper handling and disposal of pesticide wastes can create hazards for both humans and the environment. Intensive farming and pest control activities are often based on heavy pesticide use which promote waste build-up in many phases of pesticide handling (Damalas et al., 2008). Countries throughout the world face the problem of pesticide waste management, although the nature of the problem and therefore the focus of attention differ between highly industrialized and developing countries. In industrialized countries, pesticide waste management has focused on handling of wastewater, disposing and recycling of used containers, and remediation of contaminated soils and effluent (Felsot et al., 2003). In developing countries, disposal of used, unwanted, or obsolete pesticide stocks is still a major problem due to lack of the necessary state-of-the-art technologies required for pesticide disposal, which has resulted in contaminated soils, surface water and groundwater (Jain, 1992).

A pesticide is any substance or mixture of substances used for preventing, destroying, or mitigating any pest or weed (Arias-Estévez et al., 2008). The term pesticide will be used throughout this thesis and refers to any synthetic organic compound such as herbicides, fungicides, insecticides, rodenticides, molluscides, nematicides, plant regulators, and others (Aktar et al., 2009).

By nature, pesticides are meant to be lethal to the target pest(s) (weeds, bacteria, viruses, insects, fungi, etc.) but not to non-target species and humans (Carvalho and Hance, 1993; Aktar et al., 2009). Unfortunately, this is not always the case as some reports point to the negative impact of pesticides on human health and the environment if inadequately managed (Williams et al., 1999; Ayranci and Hoda, 2004; Kyriakopoulos and Doulia, 2006; Issa et al., 2010). The negative impact of pesticides on human health is well documented. Chronic respiratory disease (asthma) in Lebanon was linked to pesticide exposure in children (Salameh et al., 2003). In the US, cancer mortality was significantly increased, particularly nervous system cancers and lymphatic/hematopoietic cancers, in populations exposed to pesticide (Flaming et al., 2003), while Garry et al. (1996) linked birth anomalies (circular/respiratory, urogenital, and

musculoskeletal/integumental) to pesticide appliers. Studies carried out in Spain (García-Rodríguez et al., 1996) also linked cryptorchidism in male children to long-term exposure of their mothers to pesticides and, in New Zealand, Hanify et al. (1981) found a positive correlation between birth malformations (defects of the heart, hypospadias, epispadias, talipes) and long-term exposure to the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and an impurity 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

The concentration of each pesticide that will affect human health is calculated based on a default exposure assumption. The calculated concentration is referred to as Human Health Benchmark for Pesticides (HHBP). The following equation can be used to calculate chronic (lifetime) exposure for any particular pesticide (EPA, 2013).

$$Chronic\ HHBP = \left[cRfD\left(mg\ kg^{-1}\ bw\ day^{-1}\right) * BW(kg) * 1000(mg\ mg^{-1}) * 0.2\ RSC\right] / \left[DWI(L\ d^{-1})\right]$$

[Eq. 1.1]

Where: cRfD = chronic reference dose, BW (body weight) = 70 kg for the general population and 66 kg for females (13-49 years), RSC = Relative Source Contribution assumed as 20% and DWI = Drinking Water Intake.

Pesticides can negatively impact the environment by contaminating soils, surface water and groundwater (Laroche and Gallichand, 1995; Kopling et al. 1998; Scribner et al., 2000; Kristensen et al., 2001; Waite et al., 2002; Donald et al., 2007) and exposure to non-targeted organisms such as plants, earthworms, termites, ant colonies, snakes, birds, toads, lizards, (Macharia et al., 2009). However, inspite of these reports on the adverse effects of pesticides, the global market value for pesticides increased from $900 million in 1960 (Pingali and Gerpacio, 1997) to nearly $40 billion in 2007 (EPA, 2007). The use of pesticides in agriculture has resulted in increased crop yields and food quality globally (Warren, 1998; Ecobichon, 2001; Kumar et al., 2007). However, other factors such as improved varieties with high yielding potential, fertilizers, development of water resources, intensive cropping, and use of machinery also have contributed to this increase in productivity (Khan et al., 2010).

Not long after the use of pesticides became widespread, concerns arose about their potential adverse impact on the environment. Environmental studies conducted in the late 1950s and early 1960s found water bodies and microorganisms contaminated with the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and the insecticide dichlorodiphenyltrichloroethane (DDT) (Carson, 1962). Since then, the debate on the risks and benefits of pesticides has not ceased and a large body of research has focused on the impact of pesticides on the environment. Pesticides are believed to enter surface water and groundwater through diffuse or point sources, with point-source contamination accounting for 40 to 90% of surface water and groundwater contamination (Debear and Jaeken, 2006; De Wilde et al., 2010).

In Canada, 41 million kg active ingredient (a.i.) of pesticides was sold in 2009 (CropLife Canada, 2010) with revenues of more than $1.9 billion. Herbicides, fungicides, insecticides, and other specialty products accounted for 76, 13, 3, and 8% of all pesticides sold in Canada (CropLife Canada, 2010). The agricultural regions of Manitoba, Saskatchewan, and Alberta are responsible for over half of the total pesticide use in Canada with Saskatchewan accounting for more than 46% of all the herbicide used in the Prairies (Waite et al., 2005). Every year, an estimated 37,000 to 500,000 wetlands in Saskatchewan may be contaminated with herbicides (Gaultier et al., 2009). This is of great concern because Prairie wetlands support 50 to 80% of North America's continental waterfowl population and produce about 50% of the primary species of game ducks (Gaultier et al., 2009). Furthermore, there are more than 100,000 farm dugouts (ponds) used by rural residents as potable water and household water, livestock watering, and irrigation in the Canadian Prairies (Cessna and Elliott, 2004). The challenge for Saskatchewan and the Prairies in general is how to protect surface water and groundwater from contamination as a result of the use of pesticides.

Three types of remediation techniques namely, bioremediation (bio-stimulation, bio-augmentation, and phytoremediation), chemical (electron-beam irradiation, mercury extraction, rediocolloid treatment, and sorption to organo-oxide), and physical (air sparging/air stripping and incineration) have been used to de-contaminate the environment and polluted water destined for human consumption (Hamby, 1996; Tortella et al., 2010). However, some of these remediation techniques are expensive for the local

farmer, and as a result, bioremediation is the typically the preferred choice for on-site treatment of effluent contaminated with pesticides (De Wilde et al., 2007).

Bioremediation is a technology that destroys various contaminants using natural biological microorganisms. It is a relatively low-cost, low technology technique that can be carried out on-site (Vidali, 2001). One such bioremediation technique is the biobed. Biobeds were introduced in Sweden in 1993 to reduce surface water and groundwater pollution arising from point-source contamination by pesticides (Torstensson and Castillo, 1997). A number of field and laboratory studies conducted across Europe and Latin America with biobeds indicate their potential to render harmless pesticides and their metabolites (Castillo et al., 2001; Fogg et al., 2004a; Spliid et al., 2006; De Roffignac et al., 2008; Karanasios et al., 2010a).

Biobeds are not established in Saskatchewan nor Canada except for limited research purposes. Their performance in relation to Saskatchewan soil type and climatic condition is uncertain. Therefore, understanding the effects of temperature, moisture and composition using locally available material is needed to better understand biobed functionality and adaptability in Saskatchewan, in particular, and Canada in general.

The biobed concept has generated interest around the world. Its acceptance in some countries has seen the name and design changed or modified. For example, modified versions of the original Swedish below-ground biobed (BGB) are called Phytobac® and Biobac in France, Biofilters in Belgium, and Vertical Green Biobed in Switzerland, (Castillo et al., 2008). In Saskatoon, a new biobed design called the above-ground biobed (AGB) is under investigation. Water management in the biobed is a concern as it could affect its performance. To understand which biobed will better manage moisture, the AGB was compared with the BGB.

Carbon dioxide (CO_2) and microbial activity measurements have been used to measure the impact of pesticide addition on the microbial biomass carbon (MB-C) in the biobed (Henriksen et al., 2003; Coppola et al., 2007; Vischetti et al., 2007). However, none of these studies linked the degradation of the studied pesticides to CO_2 evolution and/or microbial activity. This aspect will be investigated in this study.

1.1 General Objectives of this Study

The general objectives of this study were to:

1. Investigate if there is any correlation between a.i. breakdown and CO_2 emission as an indicator of microbial activity in a biobed mix and topsoil.
2. Study the degradation of seven pesticides in a biobed mix under three temperatures likely to be encountered in Saskatchewan.
3. Compare two field designs of biobed to pesticide leaching potential, pesticide degradation, water balance, and temperature.

This thesis consists of six chapters. Chapter 2 summarizes and discusses pertinent literature on pesticide regulation in Canada, sources of surface water and groundwater pollution, factors affecting pesticide degradation in soil and biobed mix, types of biobeds, and government regulations of biobeds. Chapter 3 investigates the relationship between CO_2 emission as an indictor of microbial activity and a.i. breakdown at two temperatures using both biobed mix and topsoil. Chapter 4 discusses the effect of temperature on pesticide degradation. Chapter 5 compares two designs of field biobed located at the Agriculture and Agri-Food Canada (AAFC) experimental research farm in Saskatoon, SK. Chapter 6 consists of the general discussion of this study with emphasis on the applicability of biobed use under Saskatchewan climatic conditions, recommendations and potential future research areas.

2.0 LITERATURE REVIEW

2.1 Pesticide Regulation in Canada

Pesticides are stringently regulated in Canada through a program of pre-market scientific assessment, enforcement, education, and information dissemination. These activities are shared among federal, provincial/territorial and municipal governments, and are governed by various Acts, regulations, guidelines, directives, and by-laws with the goal of protecting Canadians from undue risk posed by use of pesticides (Health Canada, 2011).

At the federal level, the Pest Management Regulatory Agency under the supervision of Health Canada is responsible for pesticide registration, re-evaluation, Pest Control Products Act and regulation, human health and safety, environmental impact, compliance, and enforcement. The provincial/territorial governments are responsible for transportation, sale, usage, storage/disposal, training/certification, and licensing of applicators/vendors, spills/accidents, permits/use restrictions, compliance, and enforcement. Municipalities are responsible for by-laws (and, in some cases, private/residential) lands only (Health Canada, 2011). Pest Management Regulatory Agency is mandated to ensure that any registered pesticide in Canada poses minimal health risk to humans, and the environment. There are over 7,000 pesticide products and approximately 500 a.i. with registered use in Canada, mainly in agriculture (91%). In 2009, Canadian agriculture used 41 million kg a.i. (CropLife Canada, 2010).

The Pest Management Regulatory Agency has effectively reduced environmental and human health problems associated with pesticide usage (Sangodoyin and Smith, 1996) by identifying some principal factors (soil type, agricultural practices, meteorological information and topography) believed to influence the mobility and persistence of pesticides in the soil environment and groundwater (Crowe and Booty, 1995). Even with tight regulations on pesticide registration, numerous studies conducted across Canada found pesticide residues in surface water and groundwater (Goss et al., 1998; Waite et al., 1992; Cessna and Elliott, 2004; Waite et al., 2005; Shymko and Farenhorst, 2008; Gaultier et al., 2009). An extensive study conducted across the Northern Great Plains of North America examining 45 pesticides in reservoirs receiving water primarily from snowmelt and rainfall runoff from agricultural crop fields, detected

two insecticide and 27 herbicide and metabolites (Donald et al., 2007). A study of 1,300 wells in rural Ontario found six wells contaminated with pesticide residues above the interim maximum acceptable concentration of 0.1 μg L^{-1} (Goss et al., 1998). Considering that 30% of Canadians depend on groundwater for their domestic needs (Statistics Canada, 1996), these findings and others emphasize the need to develop alternative methods for handling pesticides and pesticide wastes in and around farmlands.

2.2 Sources of Surface Water and Groundwater Contamination by Pesticides

Research has identified a number of potential entry routes of pesticides into surface water and groundwater. The nature and properties of the a.i. and prevailing agro-climatic conditions will determine the fate of the pesticide once introduced into the soil environment (Carter, 2000). Once surface water and/or groundwater are contaminated with pesticide, it might take months to years to clean up and there is always the risk of bioaccumulation by plants and other microorganisms (Dalvie et al., 2003). The fate and behaviour of pesticides in the soil environment is determined by factors such as runoff, volatilization, photodecomposition, plant uptake, chemical transformation, and biological degradation (Figure 2.1) (Sarmah et al., 1998). Generally, it is believed that pesticides enter surface water and groundwater through two mechanisms; namely point and diffuse sources.

2.2.1 Point-source contamination

Point-source contamination is any type of contamination arising from a localized point(s). It is considered a major source of pollution and accounts for 40 to 90% of surface water and groundwater contamination (De Wilde et al., 2010). In farmyards, point-source contamination includes spills occurring during sprayer filling, leakage from faulty equipment such as sprayers, internal and external sprayer cleaning, spray leftovers, and waste disposal (Carter, 2000). Studies conducted in Germany, Sweden, Denmark, the UK, and the US all identified point-source contamination as the most important source of groundwater pollution by pesticides in and around loading areas (Spliid et al., 2006; Castillo and Torstensson, 2007). Loading sites are generally characterized by the removal of the topsoil layer (rich in organic matter and microorganisms) and replaced with either a concrete pad, a layer of sand, and/or gravel. This offers little opportunity for pesticide

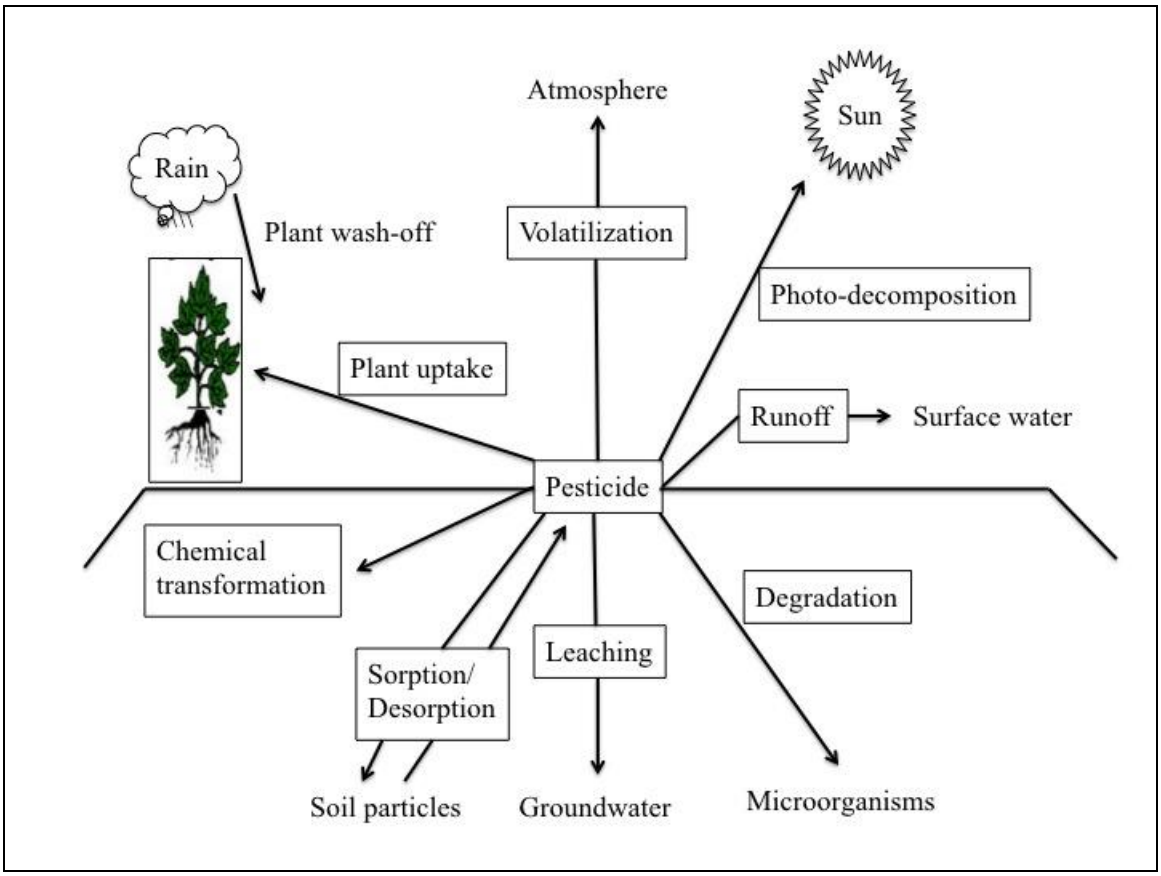

Figure 2.1 Processes that influence the behaviour and fate of pesticides in the environment (adapted from Rao et al., 1983 and Sarmah et al., 1998).

sorption and subsequent degradation by soil microorganisms (Fogg et al., 2004a,b). Hence, any accidental spill of a.i. could easily find its way into the surrounding waters.

2.2.2 Diffuse-source contamination

Diffuse-source pollution or non-point source refers to contamination in which neither the source nor the size of the specific emission observed is readily identifiable. In the case of pesticides, diffuse-source pollution involves the movement of pesticides from the field of application to surface water and groundwater through mechanisms such as surface runoff, leaching, flow/interflow lateral drainage, volatilization, precipitation, and spray drift (Carter, 2000; Fait et al., 2007; Spanoghe et al., 2004).

2.3 Factors Affecting Pesticide Degradation in Soil

Soil is a unique part of the natural and agricultural aspects of the terrestrial ecosystem given its role in plant growth, degradation, and recycling of dead biomass (Pavel and Gavrilescu, 2008). When pesticides are introduced to the soil environment, they undergo complex interactions involving physical, chemical, and biological processes (Ghafoor et al., 2011). These processes are governed by the physicochemical properties of the pesticide such as molecular weight, ionizability, water solubility, lipophilicity, polarity, volatility, soil chemical composition (organic matter, pH, nutrient availability), environmental conditions (moisture, temperature, and aeration), and the presence of microorganisms (Walker et al., 2000). However, only one or two properties have a dominating influence (Gevao et al., 2000).

Pesticide mobility is not a good indicator for determining the potential negative impact of a pesticide in the soil environment. However, a combination of mobility and persistence will determine if a pesticide will be degraded during its residence time in the soil zone above the groundwater. Gustafson (1989) proposed a single numerical index for predicting a pesticide's potential for contaminating groundwater, the groundwater ubiquity score (GUS). It is defined as:

$$GUS = Log\left(T_{1/2}^{\ soil}\right) * \left(4 - \log\left(K_{oc}\right)\right) \qquad [2.1]$$

Where ($T_{1/2}^{soil}$) is the half-life of the pesticide in soil and K_{oc} is the partition coefficient of the pesticide between soil organic carbon (SOC) and water. Pesticides with a GUS value greater than 2.8 were generally detected in groundwater while those with a GUS value less than 1.8 were not (Gustafson, 1989).

Degradation (half-life) and sorption are the two key processes used to predict the impact of a pesticide in soil and losses to surface water and groundwater (Arias-Estévez et al., 2008; Villaverde et al., 2008). The loss of pesticide in soil through microbial and chemical pathways is termed degradation and is influenced by: 1) physiochemical properties of the soil (pH, organic matter, clay content, mineral ion content, degree of aggregation), 2) biological properties (distribution of microbial populations and activities), 3) abiotic factors (soil temperature, soil moisture content, and air movement), and 4) chemical properties of the pesticide (stability of the parent compound or metabolites, volatility, solubility, formulation) (Edwards, 1975; Kah et al., 2007).

2.3.1 Physiochemical properties of soil

Retention and mobility of a pesticide are determined by the extent and strength of sorption reactions governed by the physical properties of the soil and the pesticide. The nature or source of organic matter (OM) has little effect on the sorption process. Pesticide mobility in low OM soils is often related to the inorganic portion of the soil, which is predominantly the clay fraction (Spark and Swift, 2002). Generally, sorption limits the degradation of pesticides by reducing their partitioning between the solid-liquid phases by forming bound residues. This makes the sorbed pesticide less accessible to microorganisms that can use the pesticide exclusively or preferentially in solution as a source of C or energy (Guo et al., 2000).

Soil type plays an important role in determining whether or not a pesticide will be adsorbed. Soil structure (pore size and distribution) affects pesticide leachability as it controls water movement (hydraulic conductivity) through the soil profile (Edwards, 1975). If the soil has a coarse sand or coarse loamy sand texture, water movement will be rapid due to the large pore size. However, if it has a clay texture, the hydraulic conductivity will be significantly reduced due to the small pore sizes. Reduced water flow down the soil profile, could result in slow movement of the pesticide, a

characteristic of soils with high clay content. This slow movement could result in more degradation of the pesticide if not adsorbed onto the soil particles. Clay soils have a minor effect on the leaching of sulfonylurea herbicides, because these herbicides are weak organic acids that are anionic under field conditions and are repelled by largely anionic clay surfaces (Cranmer et al., 1999). Preferential water flow in soil or any other media is a process in which infiltrating water by-passes the soil matrix, resulting in more rapid and deeper movement of water and solutes than would otherwise be expected (Ogawa et al., 2002). Preferential water flow allows much faster transport of water and solutes, there-by creating a greater risk of groundwater contamination (Bauters et al., 2000).

Soil pH, OM, and clay are believed to have the greatest influence on the fate of pesticides in soil. For example, the degradation of the insecticide chlorpyrifos is pH-dependent. Half-lives of 256, 58, 35, and 16 d were reported at pH 4.7, 5.7, 6.7, and 7.7, respectively, in five UK soils and one Australian soil (Singh et al., 2003). Soil organic matter can affect the degradation and leaching of pesticides. For example, soils with high OM have greater 2,4-D sorption and degradation compared to those with less OM (Rodríguez-Rubio et al., 2006).

2.3.2 Biological properties (distribution of microbial populations and activities)

Mineralization (the complete breakdown of organic molecules such as pesticides in soil) is usually due to the activities of microorganisms. Many pesticides are degraded through co-metabolism mechanisms with the organisms responsible for the degradation apparently showing no capacity to proliferate following degradation of the compound. Other pesticides are degraded by growth-linked metabolism, in which organisms responsible for their degradation have adapted to use the pesticide as an energy and C source, resulting in cell proliferation and increase in degradation rate (Alexander, 1981; Bending and Rodríguez-Cruz, 2007).

Among the various groups of microorganisms (bacteria, fungi etc.) found in soil, fungi are unique because they secrete a variety of extracellular enzymes with non-specific modes of degradation. This allows fungi to degrade both soluble and insoluble contaminants. Several species of bacteria are capable of degrading a wide range of toxic

pesticides (Osman et al., 2008). The presence of a particular pesticide in soil will determine the activity of the microorganisms by either increasing or decreasing their number. If the pesticide acts as a source of C and energy for the microorganisms, their activity will increase. However, if the pesticide is toxic to the microorganisms then their activity will be reduced, and there is an expectation that the rate of degradation of the pesticide will also decrease (Bolan and Baskaran, 1996). However, microbial communities are composed of several species likely to be responsible for the degradation of pesticides in soil rather than a single species. This implies that a complete stop to microbial activity at any point in time is very unlikely. Generally, herbicides appear to have no adverse effect on the total population of soil bacteria except at concentrations higher than field recommended rates, and fungi are not as susceptible to herbicides and insecticides as they are to fungicides (Digrak and Özçelik, 1998).

Pesticide degradation in soil is thought to occur in the top few centimeters and is believed to decrease down the soil profile (Fomsgaard, 1995). In some instances, however, degradation rates of pesticides susceptible to both co-metabolic and growth-linked degradation can be greater in subsoil than in topsoil. The precise relationship between top- and sub-soil degradation rate can vary between different compounds at a single site, or at different sites for individual compounds (Di et al., 1998; Karpouzas et al., 2001; Mills et al., 2001). The reasons for the contrasting patterns of degradation in sub-soil and topsoil are unclear.

2.3.3 Temperature and moisture

Temperature and moisture are the principal environmental factors that influence pesticide behaviour in soil. The influence of either temperature or moisture depends on the pesticide in question and soil type. There are conflicting reports on the effect of both on pesticide degradation. A study of five triazole fungicides found a 3-fold increase in degradation rate when temperature was increased from 5 to 18 ^{o}C, while a decrease in moisture content (100 to 60% field capacity) only slightly slowed the degradation rate (Bromilow et al., 1999). Blumhorst (1996) observed an increased degradation rate as temperature increased from 30 to 40 ^{o}C with increasing moisture up to field capacity.

2.4 Soil Microbial Biomass and Carbon Dioxide Production

Soil is the habitat for various microorganisms responsible for a wide range of functions essential for normal soil health. Soil microorganisms are responsible for the decomposition of OM, release of nutrients to plants in available forms and the degradation of toxic residues. Soil organic matter, total N, microbial biomass-C (MB-C), and microbial biomass-N (MB-N) are some of the soil properties used as basic indicators in assessing soil quality (Adeboye et al., 2011).

Soil microbial biomass (MB) is an important component of soil health. It is the living pool containing about 1 to 5% of the OM excluding roots and meso-and macro-fauna (De Polli et al., 2007). Microbial biomass is the main agent responsible for CO_2 production through respiration. Carbon dioxide evolution has been used to determine biological activity in soil in relation to changes in climate, physical and chemical soil properties, and agricultural practice (Sakamoto and Oba, 1994). Microbial processes affect the degradation of most pesticides in soil. Analysis of the relationship between the size and composition of microbial biomass, and pesticide degradation capacity may be useful for the assessment of pesticides applied to the soil environment. The ratio of CO_2 evolved per unit of microbial biomass is termed the metabolic quotient (qCO_2), which reflects the overall activity or energy spent by the indigenous microbial pool (Anderson and Domsch, 1990).

A positive correlation between MB, soil respiration, and the degradation rate constant of metribuzin, linuron, glyphosate, alachlor, 2,4-D, and dicamba was reported in agricultural and forest soils (Chowdhury et al., 2008). Rath et al. (1998) found a large increase in the MB when 2,4-D and its analog 2,4,5-T were applied under flooded and non-flooded conditions compared to the control.

2.5 Degradation Pathways of Studied Pesticides

2.5.1 Sulfonylurea herbicides

Degradation of sulfonylurea herbicides in soil is determined by many factors such as soil pH, temperature, moisture content, soil microbial diversity, and biochemical activity. In most cases, chemical hydrolysis is the important pathway for their degradation because these compounds have many functional groups that are susceptible

to various hydrolytic reactions. Metsulfuron-methyl and tribenuron-methyl have almost the same chemical structure with a sulfonamide and triazine amino side chains (Figure 2.2). The only difference is that in the R2 position, metsulfuron-methyl has a hydrogen (H) atom while tribenuron-methyl has a methyl (CH_3) group. Chemical degradation of the sulfonylurea herbicides is through the cleavage of the sulfonylurea bridge (acid-catalyzed and base-catalyzed contraction/rearrangement) (Andersen et al., 2001; Sarmah and Sabadie, 2002). Sulfonylurea herbicides are based on a general structure (Figure 2.2) where the R1 moiety can be either an aliphatic, aromatic, or heterocyclic group connected by the sulfonylurea bridge to the R2 moiety. The R2 position can either be a triazine or pyrimidine group. All three sulfonylurea herbicides in this study have a triazine ring in the R2 position. In the R1 position, metsulfuron-methyl and tribenuron-methyl both have a sulfonamide group whereas a thiophene group is found in thifensulfuron-methyl (Figure 2.2). Degradation of the sulfonylurea bridge is faster under acidic than alkaline conditions. The half-life of chlorsulfuron in aqueous solution at 25 ^{o}C increased from 1 to more than 500 d when the pH was increased from 3 to 7.5, respectively (Sarmah and Sabadie, 2002).

The degradation of metsulfuron-methyl in the environment is the result of both chemical hydrolysis and microbial transformation. Degradation pathways include the cleavage of the sulfonylurea bridge, *O*-demethylation of the methoxy-triazine moiety and triazine ring opening after *O*-demethylation (Wang et al., 2010). Its degradation in soil is affected by pH, temperature, and OM. Metsulfuron-methyl was found to be chemically stable at pH 7 with no detectable hydrolysis at 25 ^{o}C for 88 d, whereas at pH 5, and under the same temperature, its half-life was 33 d (González-Matute et al., 2012).

Chemical hydrolysis and microbial degradation of tribenuron-methyl in soil is through the cleavage of the sulfonylurea bridge, to form sulfonamide and triazine amino derivatives. This reaction can take place either biologically or chemically and results in the formation of two metabolites: 2-methyoxy-4-methylamino-6-methy-1,3,5-triazine and sulfonylisocyanate. Soil microorganisms play an important role in the degradation of tribenuron-methyl because more than 50% of the applied tribenuron-methyl was degraded in non-sterile soil after 4 d, while no degradation was measured in sterilized soil (Wang et al., 2011).

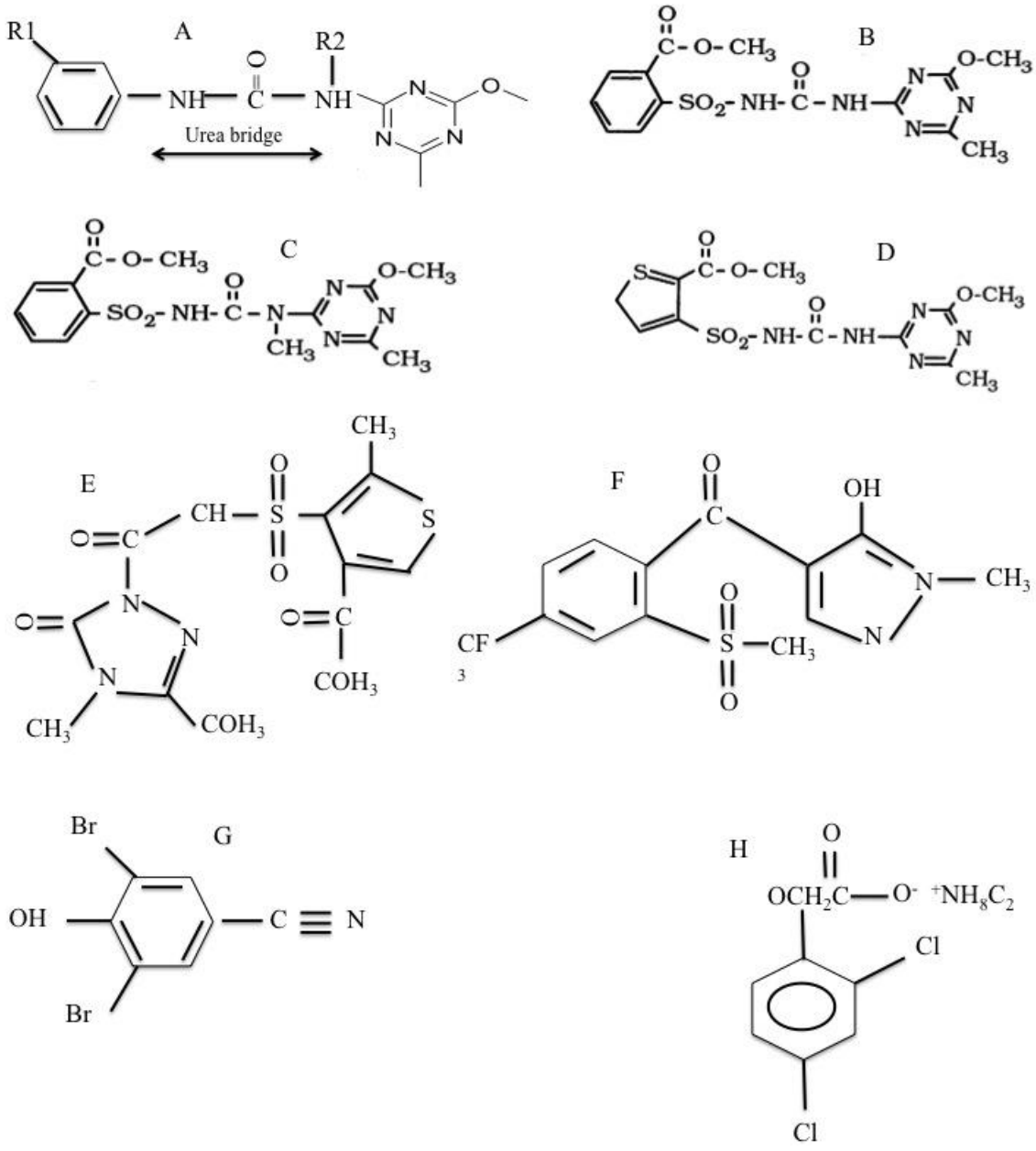

Figure 2.2 Chemical structure of studied pesticides: A) general structure of the sulfonylurea herbicides; B) metsulfuron-methyl; C) tribenuron-methyl; D) thifensulfuron-methyl; E) thiencarbazone-methyl; F) pyrasulfotole; G) bromoxynil; and H) 2,4-D dimethylamine salt.

Chemical hydrolysis and microbial degradation are the primary mechanisms through which thifensulfuron-methyl is degraded in soil and it is very susceptible to microbial degradation in soil compared to the rest of the sulfonylurea herbicides. The first step in thifensulfuron-methyl degradation begins with the hydrolysis of the ester group to form thifensulfuron acid, which is chemically inactive in soil (Cambon et al., 1998).

2.5.2 Thiencarbazone-methyl and pyrasulfotole

Thiencarbazone-methyl (Figure 2.2) is degraded in soil by microorganisms to form three major metabolites (thiencarbazone-carboxylic, thiencarbazone-sulfonamide and thiencarbazone-MMT). The parent molecule and the metabolites are persistent in soil under aerobic conditions (Health Canada, 2010). Thiencarbazone-methyl degradation in soil is influenced by factors such as moisture, soil type, temperature, and pH.

Pyrasulfotole (Figure 2.2) is degraded in soil solely by microorganisms through the cleavage of the phenyl ring to form two metabolites namely benzoic acid and pyrazole heterocycle. Factors such as OM, temperature and pH affect its degradation in soil (Kaune et al., 2008).

2.5.3 Bromoxynil

Bromoxynil (Figure 2.2) is a member of the benzonitrile herbicide group. Hydrolysis is the most common pathway for microbial degradation of bromoxynil, and this can proceed in the presence or absence of co-factors such as a C source (Rosenbrock et al., 2004). There are two possible pathways involved in the degradation of bromoxynil in soil. The first step involves a nitrile hydratase enzyme that mediates the conversion of nitrile to amide (3,5-dibromo-4-hydroxybenzamide). The amidase enzyme in turn converts the amide to carboxylic acid (3,5-dibromo-4-hydroxybenzoic acid) and ammonia. In the second pathway there is no intermediary. The nitrilase enzyme converts the nitrile directly to 3,5-dibromo-4-hydroxybenzoic acid and ammonia (Holtze et al., 2008).

2.5.4 2,4-D dimethylamine salt

2,4-D dimethylamine salt (Figure 2.2) undergoes degradation by both biological

and non-biological mechanisms in soil. Many 2,4-D-degrading microorganisms have been isolated from agricultural, urban, and industrial soils, and sediments. The catabolic pathway of 2,4-D mineralization with *Ralstonia eutropha* JMP134 (pJP4) bacteria is probably the best investigated (Maltseva et al., 1996; Marrón-Montiel et al., 2006). The bacterium uses 2,4-D as a source of C and energy. The microbial degradation of 2,4-D by *Ralstonia eutropha* JMP134 begins with the removal of the carboxyl side chain to form 2,4-dichlorophenol (2,4-DCP). 2,4-dichlorophenol is further converted to 3,5-dichlorocatechol (3,5-DCC), which is also converted to 2,4-dichloromuconate (2,4-DCM), which is also further converted to 3-oxoadipate (3-OA) and other metabolites of the tricarboxylate cycle (Leveau et al., 1999; Gaultier et al., 2008).

2.6 Techniques for Treating Contaminated Soil/Sediments

Frequent detection of pesticides and other pollutants in soil, surface water, and groundwater has led to the development of a variety of engineering-based remediation technologies that have evolved over the last three decades. These techniques can be broadly grouped into two categories: 1) isolation and containment, and 2) decontamination (Cunningham and Berti, 1993).

2.6.1 Isolation and containment techniques

Isolation and containment techniques use physical, chemical, and hydraulic barriers to isolate the pollutant and prevent its escape. A drawback to this technique is that there is no reduction in the quantity of the pollutant on a particular site. However, the risk of the contaminant(s) causing further environmental damage is reduced. Examples of containment techniques include vaults, caps, and hydraulic isolation curtains as well as physical absorption or entrapment of the contaminant into a stable matrix such as cement (Cunningham and Berti, 1993). These techniques are generally expensive and may not be applicable in some situations.

2.6.2 Decontamination techniques

Decontamination techniques (soil washing, vapour extraction and microbial bioremediation) reduce the total quantity of the contaminant at the site (Cunningham and Berti, 1993). Microbial bioremediation is a technique that transforms and/or degrades the

contaminant by stimulating the growth of microorganisms (Singh, 2008). In most cases, microorganisms use the pollutant as a source of energy. This technique has been successfully used to decontaminate groundwater, soils, lagoons, sludge, and process-waste streams (Boopathy, 2000). Today, there exist three biological techniques used for the treatment of pesticide contaminated soil and groundwater: 1) stimulation of the activity of indigenous microorganisms (bio-stimulation) through the addition of nutrients, regulation of redox conditions, and optimization of pH conditions, 2) inoculation of the site with microorganisms with specific bio-transforming abilities (bio-augmentation), and 3) utilization of plants (phytoremediation) to remove and/or transform pollutants (De Wilde et al., 2007; Tortella et al., 2010).

Activated C has been widely used to de-contaminate soil or effluent contaminated with pesticides and it is expensive (Kyriakopoulos and Doulia, 2006). However, due to their simplicity, low-cost, effectiveness and the fact that they can be carried out on site, bioremediation systems have generated considerable interest around the globe. One such technique is the biobed.

2.7 What is a Biobed?

A biobed is an inexpensive, on-farm installation intended to collect and degrade pesticides arising from filling operations, pesticide wastes, spray tank leftover, and wash residues (Torstensson and Castillo, 1997). Biobeds were introduced in Sweden in 1993 and consist of three principal components in a 60-cm deep excavation: 1) a mixture of straw, topsoil and peat in a ratio of 2:1:1 (v/v/v) referred to as biomixture, biobed mix or biomix, 2) a clay layer at the bottom (10 cm), and 3) a grass layer that covers the entire surface area (Torstensson, 2000). The biobed is equipped with a ramp for driving and positioning of the sprayer over the grassed layer.

The main function of the biobed is to reduce environmental pesticide concentration by adsorbing the pesticide on the organic components and then rapidly degrading them by the active microbial population in the biobed mix (Vischetti et al., 2004). Studies carried out in Sweden, Denmark, the UK, Italy, and Belgium have demonstrated that biobeds can effectively retain and degrade many pesticide wastes arising from accidental spill (during mixing when the sprayer is stationed on the biobed) of concentrated and prepared pesticides such as diuron, isoproturon, chlorpyrifos,

flufenoxuron, and carbofuran (Torstensson and Castillo, 1997; Torstensson, 2000; Von Wirén-Lehr et al., 2001; Fogg et al., 2003b).

2.8. Components of the Biobed

2.8.1. The biobed mix

The biobed mix is the most important component of the biobed. It promotes pesticide binding and the development of an efficient and robust microbial flora with durable pesticide degradation capacity (Castillo et al., 2008). The biobed mix composition was based on the physiological capacity of the white rot fungi (WRF) (*Phanerochaete chrysosporium*) to degrade lignin (Aust, 1995; Pizzul et al., 2009), through the action of lignin peroxidase, manganese peroxidase, and peroxide-producing systems (Castillo et al., 2001). These enzymes have been reported to degrade a variety of aromatic xenobiotic compounds such as polyaromatic hydrocarbons, polychlorinated biphenyls, and pentachlorophenol (Bending et al., 2002). The ability of WRF to degrade pesticides such as bentazon, isoproturon, metribuzin, methoxychlor, and some metabolites such as 2,5,6-trichloro-2-pyridinol (a product of chlorpyrifos degradation), has been reported (Von Wirén-Lehr et al., 2001; Pizzul et al., 2009).

Straw is the main substrate for lignin-degrading fungi, which are responsible for pesticide degradation. Straw stimulates the growth of lignin-degrading fungi such as the WRF (Castillo et al., 1997 and 2000; Von Wirén-Lehr et al., 2001). One important factor that stimulates the fungal lignin-degrading system is limitation of nutrients, especially N (Castillo et al., 2008). The nutrient status of the biobed mix needs to be known, because N can enter through the soil and compost components.

The proportion of straw in the biobed was studied but the results were inconclusive. With the exception of terbuthylazine, the degradation of metribuzin, methabenzthiazuron, metamitron, and chloridazon was correlated to the level of straw in the biobed mix (Castillo and Torstensson, 2007). Respiration rates (1.899, 0.557, and 0.432 mg CO_2 g^{-1} d^{-1}) were significantly higher in a biobed mix with high straw content (straw:peat:soil, 50:25:25 v/v/v) compared to medium (straw:peat:soil, 25:50:25 v/v/v) and low straw content biobed mix (straw:peat:soil, 12.5:62.5:25 v/v/v), respectively, incubated for 62 d at 20 ^{o}C. No significant difference was observed among the various

treatments in the degradation and mineralization of chlorpyrifos (Coppola et al., 2007).

Straw length also is an important factor in the preparation of the biobed mix. Castillo et al. (2008) used straw lengths of 5, 2, and < 2 cm to assess the degradation of several pesticides. Higher respiration rates were observed in the biobed mixes with shorter straw length; however, there was no significant difference in the degradation of the tested pesticides during 93 d of incubation.

Topsoil provides sorption and pesticide-degrading microorganisms (Vischetti et al., 2007; Spanoghe et al., 2004). Topsoil should be rich in humus with low clay content to increase bioavailability of the applied pesticide, thereby limiting possibilities for the applied pesticide to remain in micro-pores (Torstensson and Castillo, 1997).

Fogg et al. (2004a) tested various soil types: sandy loam (65.4% sand, 18.7% silt, 15.9% clay, pH 6.2, 0.9% OC); clay (19.6% sand, 36.1% silt, 44.3% clay, pH 7.3, 1% OC); and silty clay (12.9% sand, 46.5% silt, 40.6% clay, pH 7.7, 3.6% OC). Each soil type was separately mixed with peat and wheat straw in a ratio of 1:1:2 (soil:peat:straw, v/v/v). For the studied pesticides (isoproturon, chlorothalonil, and mecoprop-P), soil type had no effect on leaching losses or degradation rate and more than 98% of the applied pesticides was retained and/or degraded by each of the biobed mixes after 115 d. It was therefore recommended to use soil from the farm on which the biobed is located, because repeated applications of the pesticide(s) over time can lead to the accumulation of microorganisms adapted to degrade the applied pesticide(s).

The composition and type of OM present in the biobed mix are crucial for retention of chemicals as well as for the amount, activity, genotypic and phenotypic versatility of microorganisms responsible for the degradation of applied pesticides and breakdown of their metabolites (Castillo and Torstensson, 2007). This is because OM determines the occurrence of specific microorganisms with specific catabolic activities involved in the degradation of the applied pesticides and their metabolites (Coppola et al., 2007). However, no type of OM was recommended for use in the biobed mix.

Peat or compost also provide sorption capacity and regulate humidity in the biobed. They also decrease the pH of the biobed mix favourable for lignin-degrading fungi (Spliid et al., 2006; Vischetti et al., 2007). The degradation of terbuthylazine was correlated with the peat levels in the biobed mix ($r = 0.826$) (Castillo et al., 2008).

2.8.2 Clay layer

A clay layer at the bottom of the biobed acts as an impermeable barrier to decrease water flow out of the biobed thereby increasing the residence time of the applied pesticide(s) in the biobed. The clay layer must be wet and swollen to avoid formation of cracks that could lead to preferential flow (Castillo et al., 2008). However, Spliid et al. (2006) showed that the 10-cm clay layer at the bottom of the biobed was unable to retain all of the leachate that drained through the biobed in a full-scale model in Denmark. Fourteen percent of the most mobile pesticide (bentazone) was recovered in the leachate while the cumulative amount of the other pesticides (bromoxynil, dimethoate, fluazifop, kresoxim-methyl, MCPA, mecoprop, metribuzin, pirimicarb, propiconazole, and propyzamide) was less than 2% of the applied dose (5 g a.i.) over a period of 563 d. A possible solution is to place a roof over the biobed or increase the thickness of the clay layer.

2.8.3 The grass layer

The grass layer helps to regulate the water content of the biobed by creating an upward transport of water. It can also produce root exudates (for example peroxidases) to support co-metabolic processes, especially in the upper part of the biobed where most of the pesticides are retained and degraded (Castillo et al., 2008). The absence of a grass layer reduces evapotranspiration and this may lead to cracks at the top of the biobed that could promote drainage of water through preferential flow. This may increase the risk of applied pesticides leaching out of the biobed (Castillo et al., 2008). A study in Denmark showed that even a well-established grass layer was unable to prevent downward movement of water in the biobed during the summer period (Spliid et al., 2006).

2.9 Types of Biobed Substrates and their Effects on Pesticide Degradation

The un-availability of certain biomix ingredient(s) in some regions, has led to the testing of alternative materials to replace one or more of the original components of the biobed mix, minimize the operational cost, and adapt the biobed to local conditions (Kravvariti et al., 2010). It is however recommended that any modification of the original biobed mix component(s) should have the same properties to achieve degradation of the

applied pesticide because the biobed mix is the key factor controlling the efficiency of the biobed (Castillo et al., 2008).

Under Mediterranean conditions, peat and wheat straw are not readily available and economical to use as biobed mix material (Coppola et al., 2007; Vischetti et al., 2007). Four biobed mixes (citrus peel + garden compost, citrus peel + urban waste compost, vine branches + garden compost, and vine branches + urban waste compost) in a ratio of 1:1 (v/v) were tested in Italy. There was no significant difference in the degradation of chlorpyrifos, metalaxyl, and imazamox in the various biobed mixes (Vischetti et al., 2004, 2007). A composition of an Italian biobed mixes with vine branches, urban/garden compost, and Italian soil (2:2:1, v/v/v) was compared with a Swedish biobed mix with vine branches, peat, and Swedish soil (2:1:1, v/v/v). Both biobed mixes were similarly effective in degrading the broad-spectrum insecticide chlorpyrifos (Vischetti et al., 2007). Coppola et al. (2007) also found no significant difference in chlorpyrifos degradation between a typical Swedish biobed mix and an Italian version made with citrus peels.

In Greece, a wide range of lignocellulosic agricultural materials (sunflower crop residues, olive leaves, grape stalks, orange peels, corn cobs, and spent mushroom substrate) at various proportions has been tested as a substitute for peat. Karanasios et al., (2010a,b) concluded that a biobed mix composed of soil:compost (olive leaves):grape stalk (1:1:2, v/v/v) was the best substitute for the original Swedish biobed mix composition in Greece. The authors concluded that if peat is to be replaced by compost, the compost should have certain physiological properties including high organic C content, neutral pH, low N content and a high microbial activity (Karanasios et al., 2010b).

In France, bagasse, a residue from sugar cane production, was used as a substitute for straw. It was mixed with topsoil in a ratio of 3:1 (bagasse:soil, v/v) and the resulting biobed mix was capable of degrading more than 99% of applied malathion and glyphosate, and 90% of lambda-cyhalothrin in six months in a modified biobed called Phytobac® (De Roffignac et al., 2008).

2.10 Types of Biobeds

There are two broad types of biobeds: unlined and lined (Castillo et al., 2008).

2.10.1 Unlined biobed

An unlined biobed (Figure 2.3) has no synthetic impermeable membrane isolating it from the environment. The excavation is filled with the biobed mix and its boundary is the natural soil. In many cases, a natural clay layer is present at the bottom of the pit and if not, an artificial clay layer is added (De Wilde et al., 2007; Castillo et al., 2008). The original Swedish engineered biobed first built in 1993 falls under this category. The construction of such a system does not allow for the collection of leachate (drainage water), but removes the need for water management while maintaining near-optimal conditions for pesticide degradation (Fogg et al., 2004b). There are about 1,500 such biobeds currently in use in Sweden (Castillo et al., 2008).

2.10.2 Lined biobed

A lined biobed (Figure 2.4) is similar in design to the unlined biobed; however, the whole system is lined with an impermeable membrane (plastic or concrete) that isolates it from the environment. This design permits the collection of leachate in a near-by tank or pumping of leachate from the bottom of the biobed. To improve drainage, a drainage layer (gravel or sand) is usually installed below the clay layer (Figure 2.4). Leachate collected in a near-by tank or pumped from the bottom of the biobed is usually re-circulated back to the biobed at a later time. This design is currently used in the UK (Fogg et al., 2004c; Castillo et al., 2008). The lined biobed is considered attractive to regulatory authorities because of its potential to minimise contamination of surface water and groundwater by pesticides. The drawback of the system involves water management. The biobed needs to be covered during heavy rainfall to exclude rainwater, and leachate at the bottom of the biobed must be drained or pumped out of the system to prevent waterlogged conditions. Once covered, the top 10-cm layer dries to form a cap. The formed cap can hamper hydrological conductivity thereby severely restricting evaporation of moisture from the system. This could lead to waterlogged conditions at the bottom and reduced microbial activity within the top 10-cm layer area where most of the pesticides are retained and degraded (Fogg et al., 2004b; Castillo and Torstensson, 2007). Some form of water management can be achieved by installing a pumping system or drainage to a near-by tank but this will increase the operational cost and may render the

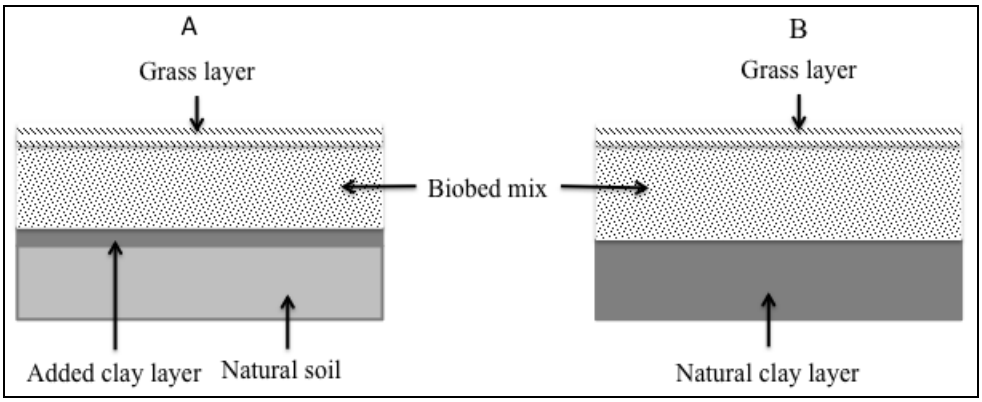

Figure 2.3 Unlined biobed a) with artificial clay layer added above natural soil, b) with natural clay layer.

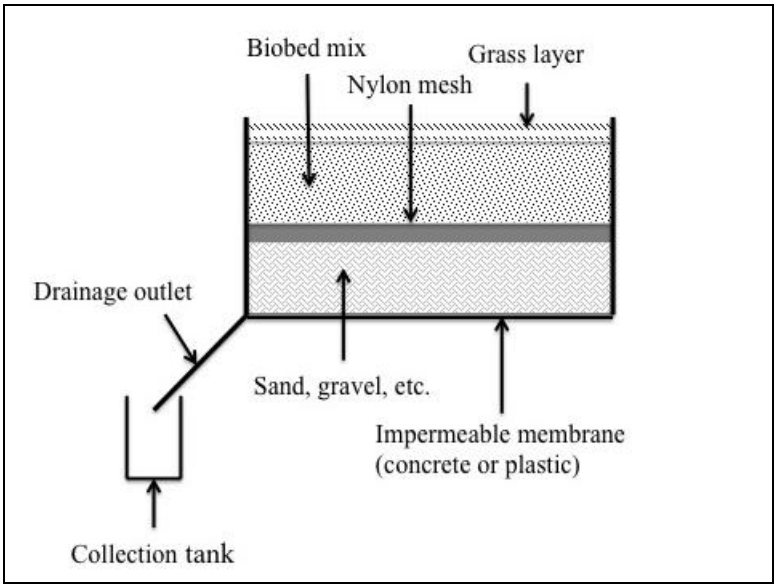

Figure 2.4 Lined biobed isolated from the environment by an impermeable membrane.

biobed less attractive to local farmers.

2.11 Engineered Biobeds

The biobed concept has generated interest around the world and its acceptance in some countries has seen the name changed and/or the original design modified (Castillo et al., 2008). For example it is called Phytobac® and Biobac in France, Biofilters in Belgium, Vertical Green Biobed in Switzerland, Biodep in Guatemala, Biomassbed in Italy, and Biobed in Sweden, the UK, and other countries. The aim of the various modifications to the basic biobed design is to adapt the biobed to various climatic conditions, availability of biobed mixture materials, and to reduce or eliminate the risk of pesticides leaching out of the system. One such modified biobed is the AGB currently being tested in Saskatoon, SK.

2.11.1 Phytobac®

The French version of the Swedish biobed (Phytobac®) is similar to the lined biobed. The agrochemical company "Rhône-Poulenc Agro France" (later "Aventis Crop Science" after merging with AgrEvo, and later acquired by Bayer and re-named "Bayer CropScience") took the initiative to experiment with the Phytobac® system (Pussemier et al., 2004). It is made up of a 60-cm deep cistern made from either concrete or plastic foil (Figure 2.5) to ensure complete retention of contaminants and effluents. The sides of the basin are 30-cm above ground level to prevent flooding from runoff water. The substrate used in this system consists of topsoil (70%) from the farm and chopped straw (30%), with no grass layer but a roof to protect the Phytobac® from rainfall (Castillo et al., 2008). Water is eliminated from the system through evaporation alone and as a result, moisture needs to be managed to avoid excessive drying and prevent waterlogging. Problems associated with use of the Phytobac® system includes 1) it requires large installations due to slow evaporation of water from the system, 2) it requires a large volume of substrate to avoid saturation or overflowing, 3) it is difficult to protect from rainfall, and 4) it is difficult to mix the substrate to obtain a homogenous mixture which is essential for pesticide retention and degradation. Furthermore, the upper layers tend to dry out quickly which could lead to hydrophobic conditions that could increase the leaching potential of applied pesticide (De Wilde et al., 2007). A study of 14 pesticides in

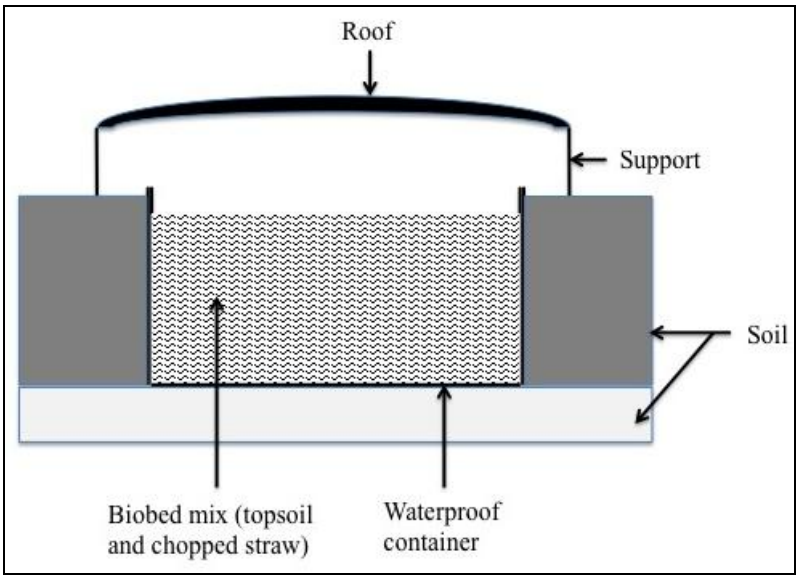

Figure 2.5 Phytobac® installation (adapted from Castillo et al., 2008).

a small-scale Phytobac® (biobed mix 1:1 v/v, soil:wheat straw) found that the system was capable of degrading the applied pesticides, with less than 1% of the applied dose remaining after two years (Table 2.1), except for terbuthylazine for which 2.3% was detected (De Wilde et al., 2007).

2.11.2 Biobac

The Biobac is another form of biobed developed in France by researchers at the National Institute of Agricultural Research. The Biobac consists of a tank buried in the soil and filled with topsoil from the farm and straw. The concept behind this system is that the farm soil is likely to have selected for microorganisms capable of degrading pesticides used on the farm. Another assumption is that the natural detoxification dynamics of the soil micro flora can be maintained with the addition of C in the form of straw (Castillo et al., 2008).

2.11.3 Biomassbed

The biomassbed was developed in Italy in 2003 with the aim of reducing pesticide contamination of surface water and groundwater. The idea with the biomassbed was to develop: 1) a functional system identical to the Swedish biobed but adapted to Mediterranean conditions, because peat is not readily available, and 2) a system capable of withstanding long dry periods without sacrificing efficiency (Fait et al., 2007). Locally available material [topsoil, green compost, and chopped vine branches (1:2:2, v/v/v)] was used for the biobed mix. The biomassbed is similar to the lined biobed but with some structural differences; e.g. the biomassbed includes a buffer tank to collect leachate. The dimensions depend on the volume of contaminated water the system will treat. Construction starts with the digging of a hole and placing a plastic tank into it. A metal grid is placed about 1 m from the bottom of the tank thereby dividing the plastic tank into two portions. The lower part is used to collect leachate, while the upper portion holds the biobed mix (Figure 2.6). Drainage is improved by lining the metal grid with nylon filter, a plastic net, and sand layer. The upper part of the tank houses an irrigation system that pumps leachate back to the biobed mix hence keeping the biomassbed moisture condition at optimal for microbial degradation. A roof is usually placed over the system to prevent

Table 2.1 Pesticide residues detected in the biobed mix (1:1 soil:straw, v/v) after 2 year (De Wilde et al., 2007).

Pesticide	Applied	Proportion remaining
	--------- g a.i.---------	----------%--------
Atrazine	2.0	-[†]
Carbetamine	5.5	-
Chloridazon	21.5	-
Chlorpropham	2.1	-
Diuron	16.2	-
Ethofumesate	5.0	0.52
Glufosinate	1.5	-
Glyphosate	16.4	-
Isoproturon	10.0	-
Isoaxben	4.0	-
Metsulfuron-methyl	0.2	-
Mesosulfuron-methyl	0.2	-
Fenmedipham	1.6	-
Terbuthylazine	16.2	2.3

[†]Measured pesticide amount below limit of detection.

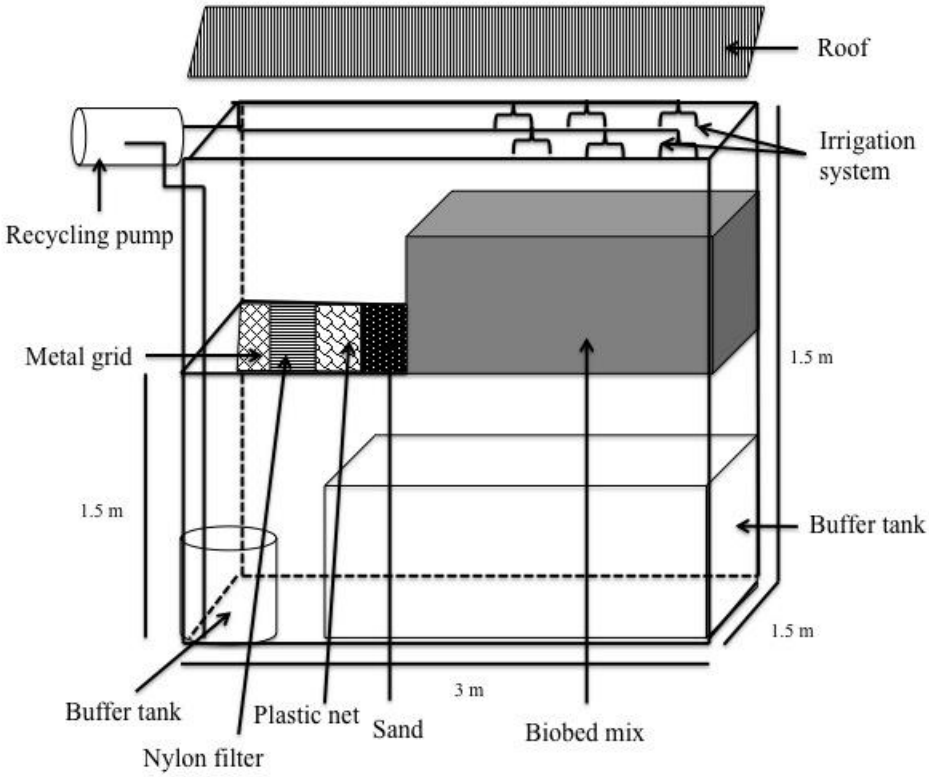

Figure 2.6 A biomassbed scheme (adapted from Fait et al., 2007).

rainwater from entering the biomassbed. Fait et al. (2007) applied cymoxanil and flufenoxuron to the biomassbed once in 2003 and chlorpyrifos once in 2004, cyprodinil, fenitrothion, fludioxinil, and iprovalicarb were all applied once in 2003 and again in 2004; metalaxyl was applied twice in both 2003 and 2004; mancozzeb and penconazole were both applied three times in 2003 and in 2004. All the pesticides, with the exception of fenitrothion and iprovalicarb (Table 2.2), achieved 92 to 100% degradation after 1 yr.

2.11.4 Biofilters

In Belgium, another modified form of the biobed, called a biofilter, has been developed and studied. It combines positive aspects of both the biobed and Phytobac® and is adapted to treat large volumes of effluent (Pussimier et al., 2004). The biofilter is made of two or three 1 m^3 plastic tanks stacked in a vertical pile (Figure 2.7) and connected with plastic valves and pipes. The bottom of each container has an internal drain towards an outflow valve to limit contaminated effluent leftover in each tank (De Wilde et al., 2007). The choice to use a two- or a three-unit system depends on the total amount of wastewater to be treated and the pesticide load. A two-unit biofilter is suited for pesticide loads ranging from 1 to 100 g a.i and a volume of less than 3000 L, while a three-unit system is recommended for higher loadings (Castillo et al., 2008). The biofilter substrate is a homogenized mixture of local topsoil, chopped straw and peat or compost. Studies show that when composted farmyard manure was used as a substitute for straw and/or peat the biofilter did not lose its adsorption or degradation properties. Recycling of the substrate after one year by mixing it with fresh organic material (straw and manure) was reported to have significantly improved the degradation of the applied pesticides (Pussimier et al., 2004). Pussemier et al. (2004) monitored the degradation of eight pesticides (atrazine, carbofuran, diuron, lenacil, simazine, isoproturon, chloridazon and chlortoluron) generated as waste after normal field applications by a farmer. After two years in the biofilter, more than 95% of the applied pesticide was degraded. The major drawback with the system is the need for constant monitoring to maintain the correct moisture and aeration levels. The lack of a grass layer may lead to preferential flow, which could lead to pesticides leaching out of the system. Biofilters are now registered with the Ministry of Agriculture and Environment of the Walloon Region in the southern

Table 2.2 Pesticide mass balance in a biomassbed filled with biobed mix (soil:green compost:vine branches, 1:2:2 v/v/v), pesticide applied in 2003 and 2004 (Fait et al., 2007).

Pesticide	Applied	Degraded	Retained (biobed mix)	Tank sediment	Water
	--g a.i.--	----------%----------			
Cymoxanil	10.9	100			
Chlorpyrifos	25	100			
Cyprodinil	24	100			
Fenitrothion	75.8	58.5	1.3	40.2	
Fludioxinil	16.2	100			
Flufenoxuron	2.6	92.6	3.8	3.6	
Iprovalicarb	14.2	25.5	72		2.5
Mancozzeb	1418.2	98			2
Metalaxyl	49.9	97	2		1
Penconazole	11.2	100			

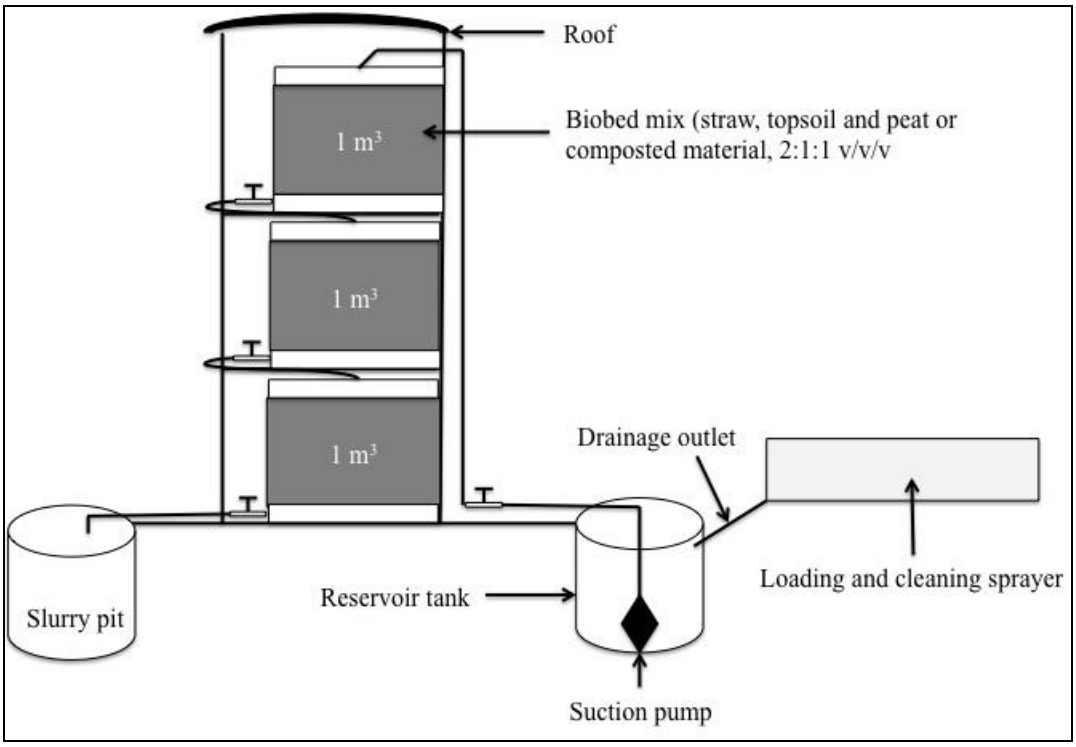

Figure 2.7 Schematic representation of the biofilter design (adapted from Pussimier et al., 2004).

part of Belgium and are recommended to pesticide users (Castillo et al., 2008).

2.12 Government Regulation of Biobeds

The introduction of biobeds in any country requires intensive research to adapt it to the country's climatic conditions and use of locally available material. This process usually involves laboratory and field studies to generate sufficient information for government approval. To date, biobeds have been approved for use in Sweden, the UK, Belgium, and France.

For biobeds to be installed in the UK, growers must register their site with the UK Environment Agency and must follow its regulations. Biobeds must be located at least 10 m away from any watercourse and 50 m away from a spring, well, or borehole (Castillo et al., 2008). Unlined biobeds may only be used for treating spills that occur during the filling, mixing, and handling of pesticides when the sprayer is placed on the biobed. If the biobed is expected to intercept equipment washing, it must be lined (Fogg et al., 2004a).

In Belgium, the focus of the biobed was a system that could effectively manage large volumes of effluent. After laboratory and field studies, biofilters are now registered with the Ministry of Agriculture and Environment of the Walloon Region in the southern part of Belgium and are recommended to pesticide users (Pigeon et al., 2005). In France, phytobec® has been approved by the French authorities.

Despite extensive laboratory and field studies conducted in Denmark, the Danish Environmental Protection Agency has classified biobeds as a potential hazard hence they are not approved for use in Denmark (Castillo et al., 2008). Although the biobed showed remarkable capacity to degrade applied pesticides, leachate analysis revealed that some pesticides were still present in the leachate after it passed through the biobed. The 0.1 µg L^{-1} limit of the European Union drinking water directive was exceeded by up to 230 and 1720 times for mecoprop and bentazone, respectively, in a 2.5 m^2 biobed at a concentration of 5 g a.i., a rate corresponding to 20 kg ha^{-1} (Spliid et al., 2006). Furthermore, Henriksen et al. (2003) reported 13 and 1.4% of applied mecoprop and isoproturon, respectively, leached out of a Danish biobed during the winter following autumn pesticide application.

Three types of bioremediation system (biobed, Phytobac®, and biofilter) have been developed so far and have proven to be highly efficient in treating pesticide wastes. For the majority of pesticides studied so far, more than 95% of the applied amount was either retained or degraded in the bioremediation system. Hence, it is strongly advisable to implement this system on farm to reduce surface water and groundwater contamination by pesticides (De Wilde et al., 2007).

3.0 DEGRADATION OF 2,4-D DIMETHYLAMINE SALT (2,4-D DMA) AND CARBON DIOXIDE EVOLUTION IN A BIOBED MIX AND TOPSOIL

3.1 Introduction

Extensive use of pesticides in agriculture is compromising soil and water quality. One major public concern is protecting water resources from pesticides and other chemicals (Boivin et al., 2005). The persistence of pesticide residues in soil depends on sorption, and the rate of chemical and microbial degradation (Bolan and Baskaran, 1996). Microbial degradation is a key factor in predicting the environmental fate of existing and newly added pesticides to the environment (Struijs and Stoltenramp, 1990; Raymond et al., 2001; Gabriela et al., 2007). Microbial biomass, the living microbial cells in the soil, is the main agents responsible for CO_2 evolution. The ratio of CO_2 evolved per unit microbial biobmass (MB) is termed metabolic quotient (qCO_2) (Sakamoto and Oba, 1994), and has been used as an indictor of the overall microbial activity before and after pesticide addition (Cortassa et al., 2001; Sengupta et al., 2009).

Pesticide degradation can be assessed either through direct measurement of the pesticide concentration or indirect measurement of the parent compound's bioconversion, using measurements such as cumulative oxygen uptake, CO_2 evolution, change in pH, decrease in dissolved organic carbon (DOC), or increase in chemical oxygen demand (COD) (Grady, 1985; Govind et al., 1997). Determination of CO_2 production has been used to quantify the biodegradation of polymer materials (Spitzer et al., 1996; Calmon et al., 2000) and pesticides (Bartha et al., 1967; Ogram et al., 1984). However, the CO_2 technique has not been fully exploited in the study of pesticide degradation in a biobed mix. A few studies have used CO_2 evolution and microbial activity to monitor the impact of pesticides on the microbial population in the biobed mix (Henriksen et al., 2003; Coppola et al., 2007; Vischetti et al., 2007), however, none of these studies linked the degradation of the studied pesticides to CO_2 evolution and/or MB. This study reports on experiments conducted to determine if there is a relationship between CO_2 evolution and a.i. breakdown in a biobed mix and in topsoil.

There are conflicting reports in the literature on the role of MB or total bacteria in soil, and OM on 2,4-D mineralization. A positive correlation was found between the size of the MB or number of total bacteria in soil, and OM (Entry et al., 1995; Voos and

Groffman, 1997). However, Entry at al. (1994) found no correlation between either active and total bacterial biomass, or active, and total fungal biomass on atrazine and 2,4-D mineralization.

2,4-dichlorophenoxyacetic acid was introduced in the 1940s and has been used for more than 60 years for post-emergent control of broadleaf weeds in cereal crops, turf, pastures, and non-cropped land (Cullimore, 1981). The ester or amine formulations of 2,4-D are widely used across Canada and it is one of the most frequently detected herbicides in soil, surface water, and groundwater in Canada (Grover et al., 1997; Waite et al., 2002; Donald et al., 2007), as well as in potable water, rain water, and farm dugouts in western Canada (Shymko and Farenhorst, 2008). 2,4-D dimethylamine salt was selected for this study because of its unique behaviour in soil. It is weakly retained in soil, vulnerable to leaching and can be degraded completely within 4 to 20 d depending on soil type and other abiotic factors (Boivin et al., 2005; Gaultier et al., 2009). Its degradation in a biobed mix had not been studied.

3.1.2 Objective

The objectives of this study were to: 1) investigate if there is a relationship between CO_2 evolution, microbial biomass-Carbon (MB-C) and a.i. breakdown in a biobed mix to which 2,4-D DMA was added, and 2) study the ability of the biobed mix to degrade 2,4-D following repeated additions of 2,4-D DMA.

3.2 Materials and Methods

3.2.1 Test chemical

The 2,4-D dimethylamine salt (560 g a.i. ha^{-1}) used in this study was donated by Bayer CropScience, Regina, SK.

3.2.2 Biobed mix preparation

The biobed mix was prepared by mixing topsoil (23 m^3), compost (23 m^3), and winter wheat (*Triticum aestivum*) straw (12 round bales ≈ 46 m^3) in a ratio of 1:1:2 (v/v/v) at the Agriculture and Agric-Food Canada research farm located in Saskatoon, SK (Brian Caldwell, personal communication). The compost (composted cattle manure) was

bought from a local farmer near Saskatoon, SK. Winter wheat straw was obtained from the University of Saskatchewan, Kernen Crop Research Farm located in Saskatoon, SK. Straw was chopped (5 to 10-cm long) with a tub grinder. Topsoil was collected from the upper soil layer (0 to 20 cm) from the Agriculture and Agric-Food Canada experimental research farm in Saskatoon, SK. Soils in this area are classified as Dark Brown Chernozem (SCSR, 1978). The three ingredients (topsoil, compost, and straw) were mixed on July 12, 2007 with a front-end loader. The mixture was turned twice with a front-end loader on August 30 and again on September 19, 2007 and was composted outside, uncovered. The chemical composition of the biobed mix, topsoil, compost, and straw was determined in 2007 and again in 2011 (Table 3.1) by ALS Laboratory Group Ltd. (Saskatoon, SK).

A portion of the prepared biobed mix and the topsoil used in the biobed mix preparation was sieved (6 mm) to remove rocks and debris. The sieved material was air-dried at room temperature for 2 wk with periodic turning.

Moisture content was determined on all biobed mix and topsoil samples by weighing 10 ± 0.03 g, drying for 24 h at 105 ± 2 ^{o}C then re-weighing. The gravimetric moisture content was calculated according to Eq. [3.1]

$$Moisture\ content\,(\%) = (wet\ sample - dry\ sample) / wet\ sample * 100\% \qquad [3.1]$$

About 10 kg of air-dried biobed mix and topsoil was placed into separate marked plastic bags and stored at 4 ± 1 ^{o}C. Prior to use, a portion of air-dried biobed mix and topsoil was removed from storage and re-hydrated in a cement mixer to a moisture content of about 25% water holding capacity (WHC). The re-hydrated biobed mix and topsoil was allowed to equilibrate at room temperature for one week with periodic turning. Pussemier et al. (2004) reported that a similar equilibration period maximized the adsorption and degradation capacity of the biobed mix.

3.2.3 pH measurement

Samples for pH measurement from the biobed mix and topsoil were oven-dried at 60 ± 2 ^{o}C for 24 h. Six grams (n=4) oven-dry weight (odw) of both biobed mix and

Table 3.1 Chemical properties of the biobed mix, topsoil, compost, and straw used in this study.

Substrates	OM[†]	Sand	Silt	Clay	C	N	WHC[‡]	C:N[§]	pH[¶]	Texture
	----------%----------									
Biobed mix[#]	9.6	27.4	43.0	29.7	4.8	0.4	41.0	10.9	7.5	Clay loam
Topsoil	1.8	9.0	52.0	39.0	6.8	0.7	33.1	10.4	7.5	Silty clay loam
Compost	13.7	60.0	18.0	22.0	9.7	1.1	59.0	8.8	nd[††]	nd
Straw	60.4	nd	nd	nd	40.5	1.5	nd	27.7	nd	nd

[†]OM = Organic matter.
[‡]WHC = Water holding capacity (w/w).
[§]C:N = Carbon to nitrogen ratio.
[¶]pH = 1:5 w/v (biobed mix or topsoil:deionised water).
[#]Data from 2011 analysis.
[††]nd = Not determined.

and topsoil were mixed with 30-mL deionised water, shaken for 30 min with a Multi Reax shaker, and allowed to settle for 30 min. A two-point calibration of the pH meter was performed at pH 4 and pH 7; pH measurements (at room temperature) were taken with an Accument pH Meter 915 (Fisher Scientific Canada Corp) 30 s after the electrode was immersed into the suspension.

3.2.4 Bioreactor design

The single 2,4-D DMA addition experiment was conducted using modified 0.5-L Mason jars as bioreactors (Figure 3.1). The Mason jars were modified in the laboratory by gluing a small (25 mL) beaker to the inside wall using Marineland® aquarium sealant and allowing the sealant to cure for 48 h at room temperature. The Mason jars were then conditioned by placing them in an oven at 50 ± 2 ^{o}C for 24 h to degas the sealant.

The second experiment involved multiple additions of 2,4-D DMA and was conducted using 250-mL biometer flasks (Bellco Glass, Vineland, NJ) as bioreactors. The biometer flask consists of a 250-mL Erlenmeyer flask fused to a 50-mL round-bottom side-arm tube (Figure 3.2). The main chamber of the flask was sealed with a rubber stopper fitted with an ascartite filter (to allow CO_2-free oxygen to enter the flask and maintain aerobic conditions); the side-arm tube was sealed with a rubber stopper pierced with a 16-gauge needle (15 cm in length and fitted with a two-way stopcock) that was used to remove and replenish the alkali solution used to trap CO_2 generated during degradation of the test substance. Biometer flasks have been used in biodegradation studies since the early 1960s (Bartha and Pramer, 1965).

3.2.5 Additions of 2,4-D DMA to the biobed mix and topsoil

Stock solutions of 2,4-D DMA were prepared by diluting 3.26 mL (single addition experiment) or 4.64 mL (multiple additions; five additions) of 2,4-D DMA product (containing 560 g a.i. L^{-1}) in deionised water to a final volume of 100 mL. The concentration of a.i. in the stock solution was 18.21 (single addition) and 25.98 (per addition for the multiple additions) mg a.i. mL^{-1}, respectively.

3.2.5.1 Single addition of 2,4-D DMA

Following the equilibration period, 35 ± 0.05 g odw of re-hydrated biobed mix or

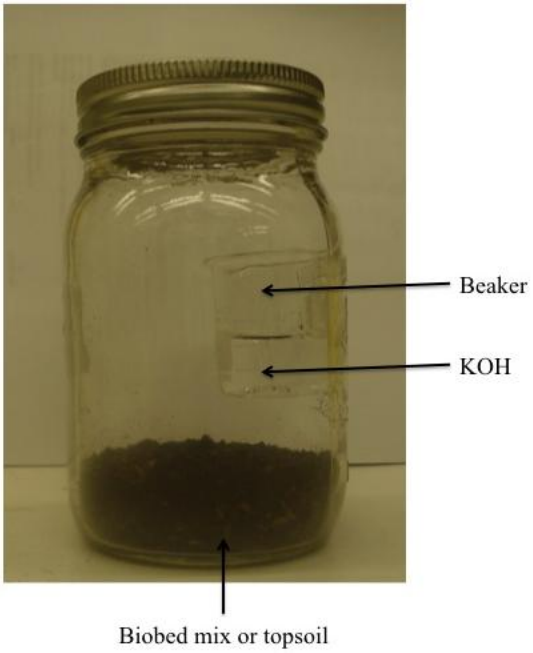

Figure 3.1 Mason jar used for monitoring CO_2 evolution from the biobed mix and topsoil.

Figure 3.2 Biometer flask used for monitoring CO_2 evolution from biobed mix and topsoil.

topsoil was measured and placed into Mason jar (0.5-L) and the water content of the matrix adjusted to 70% (w/w) WHC by adding an appropriate amount of deionised water. 1 mL of the 18.26 mg a.i. stock solution (equivalent to 512.7 mg a.i. kg^{-1}) was added to the biobed mix and topsoil while the controls received 1 mL deionised water.

In all, 48 Mason jars were prepared for the CO_2 evolution study: 24 with biobed mix to which 2,4-D DMA was added, 12 with topsoil to which 2,4-D DMA was added, 4 with just the biobed mix (i.e., biobed control), 4 with just the topsoil (i.e. topsoil control), and 4 blanks. As well, another 16 Mason jars (4 each with 2,4-D DMA added for the biobed mix and topsoil, and 4 each of the biobed mix and topsoil controls) were set aside at time-zero and placed in a freezer at -20 ± 2 ^{o}C to await pesticide residue analysis. The CO_2 trap consisted of 10 mL of 0.2 *M* potassium hydroxide (KOH) placed in the beaker (25 mL) attached to the inside wall of the Mason jar. The Mason jars were incubated at 20 ± 1 ^{o}C in the dark and the CO_2 traps changed every second day. Each time the traps were changed, the Mason jars opened (to refresh the headspace atmosphere) and weighed to determine any water loss[1].

A set of 30 additional Mason jars containing the biobed mix was set-up for the determination of MB-C: twenty-four with biobed mix to which 2,4-D DMA was added and six with just the biobed mix (i.e., biobed control).

3.2.5.2 Multiple additions of 2,4-D DMA

Following an initial equilibration period, 50 ± 0.05 g odw of biobed mix or topsoil was placed into a biometer flask and the water content of the matrix adjusted to 70% (w/w) WHC by adding an appropriate amount of deionised water. 1 mL of 25.98 mg a.i. mL^{-1} stock solution (equivalent to 519.6 mg a.i. kg^{-1}) was added to the biobed mix and topsoil, with four more additions during the 60-d incubation period (total 2,4-D DMA added = 129.9 mg a.i. kg^{-1} per 50 g biobed mix or topsoil; equivalent to 2,598 mg a.i. kg^{-1}). Following the initial 2,4-D DMA addition, the timing of subsequent additions were based on the CO_2 evolution from the biobed mix to which 2,4-D DMA was added. That is, more 2,4-D DMA was added to the biobed mix or topsoil once the CO_2 concentration

[1] Note: No significant weight (water) loss was recorded during the 34-d incubation period

trapped in the KOH dropped to the established baseline. The controls received 1-mL deionised water each time 2,4-D DMA was added to the biobed mix and topsoil.

In all, 36 biometer flasks were prepared for the CO_2 evolution study: 4 with the biobed mix to which 2,4-D DMA was added; 4 with the topsoil to which 2,4-D DMA was added; 4 with just the biobed mix (i.e., biobed control); 4 with just the topsoil (i.e., topsoil control); and 4 blanks. As well, 16 biometer flasks (4 each with 2,4-D DMA added for the biobed mix and topsoil, and 4 each of the biobed mix and topsoil controls) were set aside at time-zero and placed in a freezer at -20 ± 2 ^{o}C to await pesticide residue analysis. The CO_2 trap consisted of 10 mL of 0.1 *M* KOH placed in the side-arm section of the biometer flask. The bioreactors were incubated at 15 ± 1 ^{o}C in the dark and the CO_2 traps changed every second day. Each time the traps were changed, the biometer flasks were opened (to refresh the headspace atmosphere) and weighed to determine any water loss. The concentration of the KOH was increased from 0.1 to 0.2 *M* after 24 d of incubation. The adjustment was only made for the biobed mix to which 2,4-D DMA was added because the amount of CO_2 produced from it was higher than what the KOH (0.1 *M*) could adsorb after the second 2,4-D DMA addition and at the same time, the concentration of the hydrochloric acid (HCl) was also increased from 0.05 to 0.1 *M*.

3.2.6 Measurement of carbon dioxide production

Strotmann et al. (2004) reported that KOH was more efficient at absorbing CO_2 than sodium hydroxide (NaOH); consequently, KOH was used as the CO_2 trap in both experiments. Re-hydrating or thawing soil samples results in a flush of CO_2 evolution (Skipper et al., 1996; Lee et al., 2000). To avoid this, and achieve a stable CO_2 baseline, samples were incubated at 20 ± 1 ^{o}C (single addition experiment) and 15 ± 1 ^{o}C (multiple additions experiment) for 6 d before the addition of 2,4-D DMA or deionised water.

The KOH in the CO_2 traps was replaced every second day during the incubation. The KOH was first pipetted into a 100-mL conical flask; the trap was rinsed with 10-mL deionised water, which was added to the conical flask and the flask capped. A magnetic stirring bar was placed in the conical flask and 1 mL of 1.0 *M* barium chloride ($BaCl_2.2H_2O$) was added to the conical flask to precipitate the carbonate that had formed in the trap solution (Taok et al., 2007). A drop of phenolphthalein indicator was added to

the flask and the excess KOH was then back titrated with standardized HCl 0.1 or 0.05 *M*; single addition and multiple additions experiments, respectively. The amount of CO_2 produced was calculated using Eq. [3.2]:

$$CO_2\ evolved\ (mg\ g^{-1}\ d^{-1}) = \left[(B - V) * N * E\right] / \left(S_{odw} * t\right) \quad [3.2]$$

Where B is the titre (mL) required to titrate the blank, V is the titre (mL) required to titrate the sample, N is the normality of the HCl (0.01 or 0.05 meq mL^{-1}), E is the equivalent weight of CO_2 (22 mg meq^{-1}), S_{odw} is the oven dry weight of the biobed mix or topsoil (35 g for the single addition study and 50 g for the multiple addition study), and *t* is the time during which the trap was deployed (2 d). Percentage mineralization of 2,4-D to CO_2 was calculated using Eq. [3.3] (Vázquez-Rodriguez et al., 2008).

$$Mineralization\ (\%) = \left[\left(CO_2\right)_{2,4\text{-}D} - \left(CO_2\right)_{Control}\right] / C_{a.i.} * 100\% \quad [3.3]$$

Where $(CO_2)_{2,4\text{-}D}$ is the cumulative amount of CO_2 evolved (mg) from the biobed mix or topsoil to which 2,4-D DMA was added, $(CO_2)_{Control}$ is the cumulative amount of CO_2 evolved (mg) from the biobed mix or topsoil controls (deionised water added), and $C_{a.i.}$ is the total amount of C added as a.i.

Net CO_2 evolution was defined as the amount of CO_2 evolved from the biobed mix or topsoil to which 2,4-D DMA was added minus the amount of CO_2 evolved from the biobed mix or topsoil controls (i.e., deionised water added). For each sampling interval, the amount of net CO_2 evolved (± s.d) was calculated using the mean values (n=4) for the total CO_2 evolved from the biobed mix or topsoil to which 2,4-D DMA was added from their respective controls (El-Din Sharabi and Bartha, 1993).

3.2.6.1 Theoretical carbon dioxide production calculations

The theoretical maximum amount of CO_2 ($ThCO_2$) that could be produced during degradation of 2,4-D was calculated as using Eqs. [3.4] and [3.5] (Calmon et al., 2000).

$$C_{added}\ (mg) = (45.09/100) * (weight\ of\ a.i.) \quad [3.4]$$

and

$$ThCO_2 = C_{added} * \left(MW_{CO2} / AW_C \right) / S_{odw} \quad [3.5]$$

Where C_{added} is the amount of C added as a.i., 45.09 is the C content (%) of a.i., $ThCO_2$ is the theoretical maximum amount of CO_2 that can be produced during mineralization of the 2,4-D DMA, MW_{CO2} is the molecular weight of CO_2 (44.001 mg $mmol^{-1}$), AW_C is the atomic weight of C (12.01), and S_{odw} is the oven dry weight of the biobed mix or topsoil.

3.2.7 Determination of residual 2,4-D

3.2.7.1 Sampling and 2,4-D residue analysis

Destructive sampling of the biobed mix was carried out six times (0, 1, 3, 5, 10, and 28 d, n=4), after 2,4-D DMA addition for the single 2,4-D addition study. In the same study, topsoil sampling was performed three times (0, 10 and 28 d) n=4 after 2,4-D DMA addition. Topsoil was sampled twice after 2,4-D DMA addition because in a prior experiment there was no 2,4-D degradation within the first 5 d after addition to the topsoil.

For the multiple 2,4-D DMA additions study, sampling was performed twice: at the start of the experiment (i.e., time-zero) and upon completion of the experiment (i.e., 60 d). At each sampling time, the KOH solution was removed from both biobed mix and topsoil analysed and the biometer flask with biobed mix and topsoil immediately stored at -20 ± 2 ^{o}C. The controls (time-zero) were stored at -20 ± 2 ^{o}C immediately after the addition of deionised water to achieve a moisture content of 70% (w/w) WHC.

For extraction, bioreactors (Mason jar or biometer flask) with biobed mix or topsoil were removed from the freezer, allowed to thaw at room temperature and the biobed mix or topsoil was transferred into a 250-mL flat-bottom bottle. The samples were extracted using 20:79:1 (v/v/v) mix of water:acetonitrile:acetic acid. Samples receiving only a single addition of 2,4-D DMA were extracted using 100 mL of extraction solvent; samples receiving multiple additions of 2,4-D DMA were extracted with 150 mL of the extraction solvent. The samples were shaken for 1 h (60 rpm) with a front-end Burrell shaker and then filtered through a 0.45 µm Whatman^{TM} filter paper in a Buchner funnel

under suction. The final volume of the filtrate was adjusted to 100 mL (single addition) in a 100-mL volumetric flask or 150 mL (multiple additions) in a 200-mL volumetric flask using the extraction solvent. The solutions were thoroughly mixed and a 20-mL aliquot of the filtrate was transferred into a 20-mL glass vial and stored at 4 ± 1 ^{o}C prior to analysis.

3.2.7.2 Chemical analysis for 2,4-D

The following reagents were use for the chemical analysis. High performance liquid chromatography (HPLC) grade acetonitrile was purchased from Caledon Laboratories Ltd. (Edmonton, AB), 2,4-D analytical standard was purchased from ChemService Inc. (West Chester, PA), 2,4-D d5 internal standard was purchased from CDN Isotopes (Pointe-Claire, QC) and formic acid from Sigma Aldrich Canada Ltd. (Oakville, ON).

A Waters 2695 Alliance HPLC system with a Waters Xterra Mass C_{18} was used for 2,4-D analysis[2]. The mobile phase consisted of solvent A (90:10 water:acetonitrile) and solvent B (90:10 acetonitrile:water); both solvents contained 0.1% (v/v) formic acid. Isocratic elution of the column with 60% solvent A and 40% solvent B at a flow rate of 200 µL min^{-1} resulted in a 2,4-D retention time of 4.70 min. The injection volume was 20 µL. The 2,4-D residue was quantified using Micromass Quattro Ultima triple quadrupole mass spectrometery equipped with an electrospray ionization (ESI) interface set to negative ion mode. Ionization was optimized by infusing a 0.5 mg L^{-1} solution of 2,4-D into the ion source in a 60:40 acetonitrile:water solution with a syringe pump. Each sample and calibration standard was treated with a 2,4-D d5 internal standard to compensate for variability in the ESI process. The first quadrupole of the mass spectrometer (operating in negative ESI mode) was set to allow the parent ion through. The parent ion [219 atomic mass unit (amu)] was transferred to the second stage of the mass spectrometer where collision with helium resulted in fragmentation of the parent ion. The fragment ion (161 amu) was allowed to pass through to the third quadrupole of the mass spectrometer where the resulting ions were counted and quantified by comparison to the response given by a calibration curve made-up of a set of analytical

[2] Note: Jon Bailey performed all chemical analyses for this project at the National Hydrology Research Centre (NHRC), Saskatoon, SK.

standards. The recovery of 2,4-D was 75 ± 0.5 and 84 ± 0.8% from the biobed mix and topsoil, respectively (n=4).

3.2.8 Microbial biomass-C determination from the single 2,4-D DMA addition

Microbial biomass-C was determined in the biobed mix control (n=6) and biobed mix to which 2,4-D DMA was added (n=24) in the single addition experiment. Six replicate samples of biobed mix to which 2,4-D DMA was added were destructively sampled on Days 1, 3, 5, and 10, control samples (n=6) were sampled at time zero. At each sampling point, (except time zero), the CO_2 trap was removed and discarded, and the Mason jars were immediately stored at 4 ± 1 ^{o}C. After the last sampling point (10 d) samples from all the sampling times were extracted at the same time. Samples for MB-C were treated the same as those for residue analysis and also sampled for CO_2 every 2 d.

3.2.8.1 Fumigation treatment

Microbial biomass-C was determined using the chloroform fumigation-extraction technique (Voroney et al., 2007). Samples were removed from cold storage (4 ± 1 ^{o}C) and for each sampling time, the six replicates were split into two sets of three samples. Samples were transferred from the bioreactors into 100-mL glass bottles. Three samples were then immediately extracted with potassium sulphate solution (K_2SO_4) (0.5 *M*). The remaining three samples were subjected to a 24-h fumigation with ethanol-free chloroform ($CHCl_3$). For the fumigation treatment, samples were placed in two desiccators with the inner walls of each desiccator lined with moist paper towels; a 100-mL beaker containing 50 mL of ethanol-free $CHCl_3$ and a few boiling chips was placed in the center of each desiccator. The desiccators were connected to a 2-stage rotary pump (Cenco Hyvac 14) and evacuated until the ethanol-free $CHCl_3$ began to boil vigorously. After two minutes, the desiccators were sealed under vacuum and incubated in a fume hood at room temperature in the dark for 24 h. Following the 24 h incubation, the vacuum of each desiccator was released (in the fume hood), the beaker with ethanol-free $CHCl_3$ and moist paper towels removed and the residual ethanol-free $CHCl_3$ removed from the samples by repeated (n=5) evacuations (5 min at 10^{-5} kPa).

3.2.8.2 Extraction of microbial biomass-C

Microbial biomass-C was extracted by adding 70 mL of 0.5 M K_2SO_4 solution to the biobed mix [1:2 biobed mix (odw):extraction (v)], capping the bottles and shaking at 180 rpm on a G10 Gyrotory shaker (New Brunwick Scientific, Edison, NJ, USA) for 1 h. The slurries were filtered under suction using a Buchner funnel fitted with Whatman™ GF 934-AH filter paper. The filtrates were poured into 50-mL plastic bottles and stored at -20 ± 2 ^{o}C until analysed.

3.2.8.3 Determination and calculation of extracted carbon

Microbial biomass-C in the extracts was determined using a Shimadzu TOC-$V_{CPH/CP}$ total organic carbon analyzer (Mandel Scientific, Guelph, ON). The TOC analyzer was calibrated using a standard (50 mg C) prepared from potassium biphthalate (1000 mg C) stock solution. The calibration curve was machine generated by programming the instrument to auto-dilute the 50 mg C standard by factors of 1x, 2x, 5x, 10x, and 50x before combusting the sample. Following the removal of any inorganic C, the CO_2 produced during sample combustion was quantified using an infra-red gas analyzer.

For analysis, the biobed extracts were removed from the freezer, thawed at room temperature, shaken vigorously, allowed to settle and the supernatant transferred to plastic vials. The vials were placed in an auto-sampler and the analyzer programmed for TOC. The instrument was programmed to perform a 4-fold dilution of the sample extract prior to determining the TOC content of the sample. Two analyses were performed on each sample, with the mean value reported as sample concentration. Samples that were "out of range" were excluded. The amount of extractable C from both fumigated (C_F) and non-fumigated (C_{UF}) samples were calculated using Eq. [3.6]:

$$C_F, C_{UF}\left(mg\, g^{-1}\right) = TOC * \left(V_S - M_S\right) \qquad [3.6]$$

Where C_F and C_{UF} are the amount of extractable C in the fumigated and un-fumigated samples, respectively, TOC is the total volume of organic C in the sample extract, (i.e. extractant + water in the biobed mix), and M_S is the oven dry weight of the biobed mix.

Microbial biomass-C from the samples was calculated using Eq. [3.7]:

$$MB\text{-}\ C\left(mg\,g^{-1}\right) = \left(C_F\ \text{-}\ C_{UF}\right) / K_{EC} \qquad [3.7]$$

Where K_{EC} is the extraction efficiency for microbial biomass C; values for K_{EC} range from 0.25 to 0.45. The average value (0.35) was used (Cheng and Virginia, 1993).

3.2.9 Statistical analysis

Significant difference between the daily CO_2 production in both biobed mix and topsoil were assessed using the Student's t-test ($P \leq 0.05$); the test was performed using the online statistical program GraphPad. The MB-C data were subjected to a one-way analysis of variance (ANOVA) using the CoHort 6.4 package (CoHort 6.4 software Monterey, CA, 93940, USA).

3.3 Results

3.3.1 Carbon dioxide evolution from single 2,4-D DMA addition

All CO_2 calculations reported here exclude the CO_2 produced during the initial pre-incubation phase (6 d) during which baseline emission was established. Stable CO_2 production was achieved in both biobed mix and topsoil after 6 d of incubation without 2,4-D DMA. Two days after 2,4-D DMA addition to the biobed mix there was a slight reduction in CO_2 production from the biobed mix to which 2,4-D DMA was added compared to the control that received deionised water on Day 6 (Figure 3.3). The CO_2 level in the biobed mix to which 2,4-D DMA was added peaked on Day 12 (6 d after 2,4-D DMA addition), before returning to background levels on Day 16 (10 d after 2,4-D DMA addition). This was followed by a gradual reduction in CO_2 production until the end of the incubation period (28 d). Two days after 2,4-D DMA addition to the biobed mix and from Day 18 to the end of incubation period, after 2,4-D DMA addition, daily CO_2 production from the biobed mix control was more than that from the biobed mix to which to which 2,4-D DMA was added. The biobed mix to which 2,4-D DMA was added produced significantly higher amount of CO_2 compared to topsoil to which 2, 4-D DMA was added (Student's test, $P < 0.0001$) during 28 d of incubation.

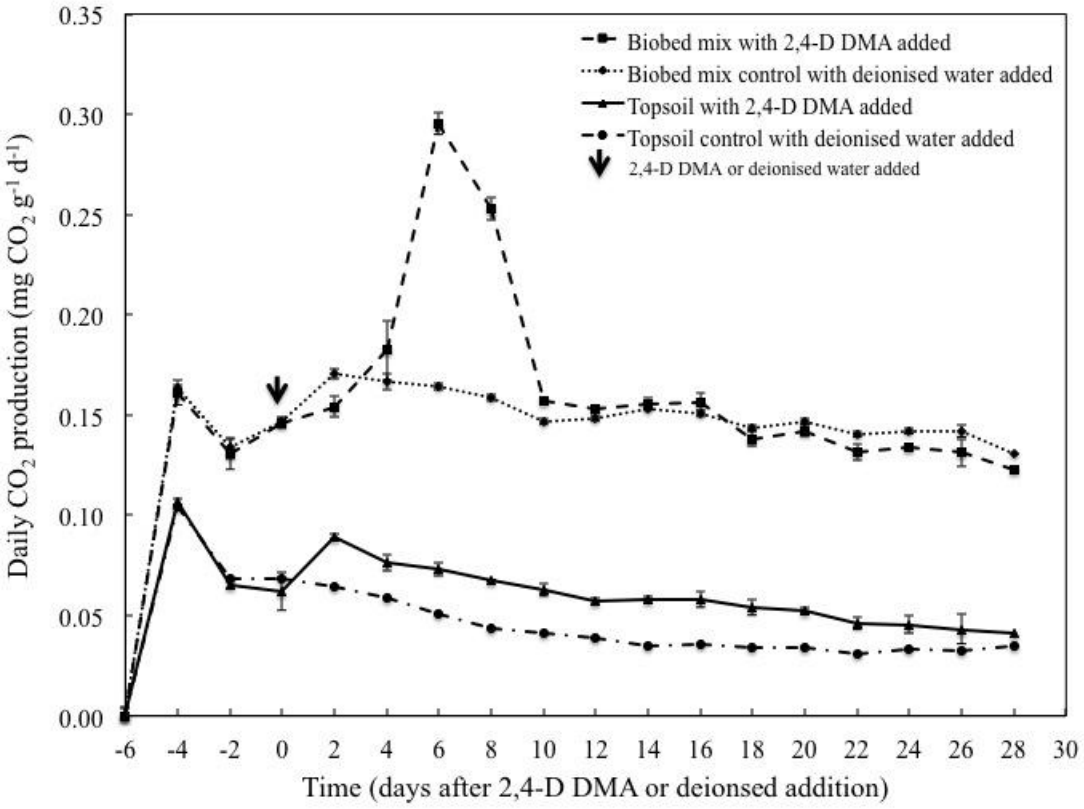

Figure 3.3 Daily CO_2 evolution per sampling time from the biobed mix to which 2,4-D DMA or deionised water (biobed mix control) was added, topsoil to which 2,4-D DMA or deionised water (topsoil control) was added in the single 2,4-D DMA addition experiment. Error bars are ± SD of the mean of 4 replicates.

In topsoil, CO_2 production was the opposite of that observed in the biobed mix. Two days after 2,4-D DMA addition there was a slight peak in CO_2 production from the topsoil to which 2,4-D DMA was added compared to the topsoil control (Figure 3.3). At no time during the study did the amount of CO_2 produced from the topsoil control exceed CO_2 produced from the topsoil to which 2,4-D DMA was added. Topsoil with 2,4-D DMA added and the control (deionised water added) produced 1.66 ± 0.10 and 1.13 ± 0.07 mg CO_2 g^{-1}, respectively, in 28 d.

Net CO_2 production in the biobed mix showed three distinct phases (Figure 3.4); Phase 1 lasted for 4 d following 2,4-D DMA addition with a net CO_2 production of -0.001 ± 0.01 mg CO_2 g^{-1}, Phase 2 also lasted for 4 d during which there was a rapid net CO_2 production of 0.226 ± 0.02 mg CO_2 g^{-1}, and Phase 3 lasted for 20 d during which CO_2 production decreased slowly and produced -0.024 ± 0.04 mg CO_2 g^{-1}. Net cumulative CO_2 production from the biobed mix because of 2,4-D DMA addition was 0.40 ± 0.15 mg CO_2 g^{-1} biobed mix, equivalent to 46.57 ± 8.88% 2,4-D DMA mineralization. About 60.26 ± 3.85% 2,4-D DMA mineralization occurred during Phase 2 (Figure 3.4).

Net CO_2 production in topsoil was continuous and linear after the addition of 2,4-D DMA. Net cumulative CO_2 production was 0.52 ± 0.03 mg CO_2 g^{-1} topsoil, after 2,4-D addition equivalent to 60.26 ± 3.85% 2,4-D DMA mineralization. A significant difference (Student's test, $P < 0.05$) was observed between net 2,4-D mineralization in the biobed mix and topsoil. Net cumulative CO_2 production from topsoil was higher than that produced from the biobed mix (Figure 3.4).

3.3.1.2 Degradation of 2,4-D DMA single addition

The 2,4-D DMA degraded faster in the biobed mix than topsoil. No significant 2,4-D degradation occurred during the first 5 d after 2,4-D DMA addition to the biobed mix. By Day 10, more than 99% of applied 2,4-D DMA was degraded (Figure 3.5).

In topsoil, 25 and 35% of the applied amount of 2,4-D DMA was degraded in 10 and 28 d, respectively, after addition (Figure 3.5). The degradation data for topsoil in single 2,4-D DMA experiment did not correlate with the net CO_2 production. Therefore, the high mineralization observed could not be attributed to microbial breakdown of 2,4-D DMA alone. Perhaps some mineralization of dead microorganisms occurred.

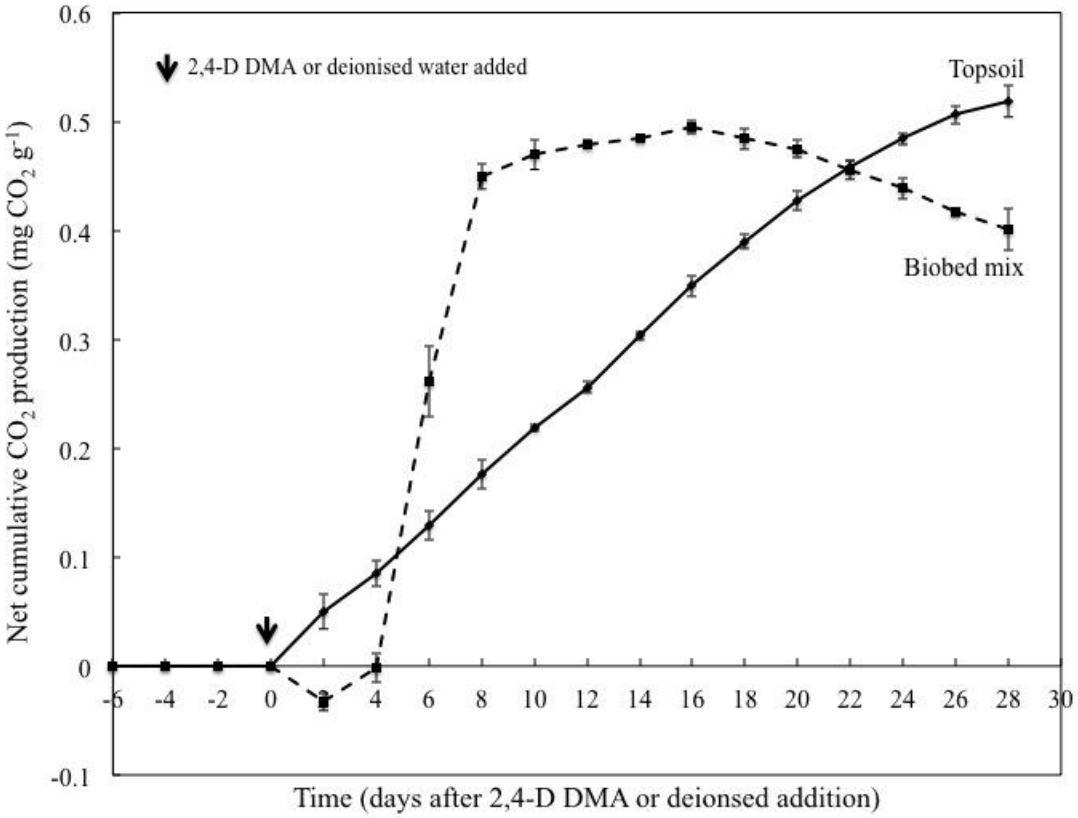

Figure 3.4 Net cumulative CO_2 production (mg CO_2 g^{-1}) from both biobed mix and topsoil with 2,4-D DMA added minus their respective controls for single 2,4-D DMA addition incubated at 20 ± 1 ^{o}C. Arrow indicates the addition of 2,4-D DMA. Error bars are ± SD of the mean of 4 replicates.

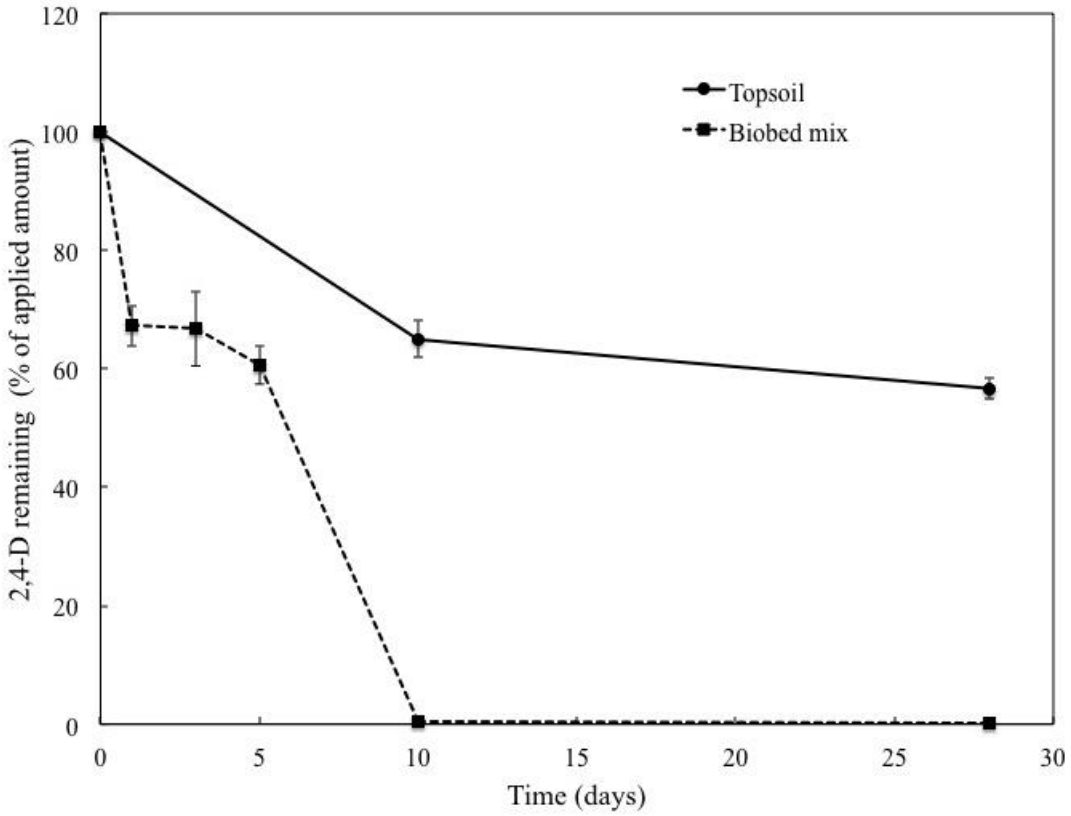

Figure 3.5 Degradation of 2,4-D in both biobed mix and topsoil incubated at 20 ± 1 ^{o}C for 28 d after 2,4-D DMA addition. Error bars are ± SD of the mean of 4 replicates.

3.3.1.3 Microbial biomass-C from biobed mix to which 2,4-D DMA was added single addition

Microbial biomass-C was measured during a period of 10 d in the biobed mix to which 2,4-D DMA was added (Figure 3.6). There was no significant difference in the MB-C among the sampling dates (ANOVA, $P \leq 0.05$).

3.3.2 Carbon dioxide evolution from multiple 2,4-D DMA additions in both biobed mix and topsoil

The biobed mix to which 2,4-D DMA was added (multiple additions) produced 9.47 ± 0.35 mg CO_2 g^{-1} biobed mix during 60 days of incubation. At the same time, the biobed mix control produced 5.44 ± 0.14 mg CO_2 g^{-1} biobed mix. A lag phase was observed in the biobed mix following the initial 2,4-D DMA addition (Figure 3.7). However, subsequent additions to the same matrix produced an immediate release of CO_2. The amount of CO_2 produced from the biobed mix to which 2,4-D DMA was added was constant after the initial 2,4-D DMA addition.

The topsoil to which 2,4-D DMA was added and deionised water (control) produced 3.66 ± 0.18 and 1.16 ± 0.07 mg CO_2 g^{-1} topsoil during 60 days of incubation, respectively. There was a gradual increase in CO_2 production from the topsoil to which 2,4-D DMA was added with each addition. However, a sharp increase was only noticed after the fourth and fifth additions (Figure 3.7). Following the fifth addition, the amount of CO_2 produced from the topsoil to which 2,4-D DMA was added was slightly higher than that from the biobed mix control (Figure 3.7). Total CO_2 produced from the biobed mix and topsoil to which 2,4-D DMA was added differed significantly (Student's test, $P < 0.0001$).

During 60 days of incubation following the equilibration period, the biobed mix produced a net 4.03 ± 0.01 mg CO_2 g^{-1} biobed mix equivalent to 93.71 ± 0.30% 2,4-D DMA mineralization. The efficiency of the biobed mix was unaffected by repeated application of 2,4-D DMA. The topsoil produced a net 2.20 ± 0.01 mg CO_2 g^{-1} equivalent to 51.27 ± 0.16% 2,4-D DMA mineralization over the same period. The efficiency of the topsoil to degrade 2,4-D DMA increased with increased exposure compared to single addition experiment. The net CO2 production from the biobed mix was significantly

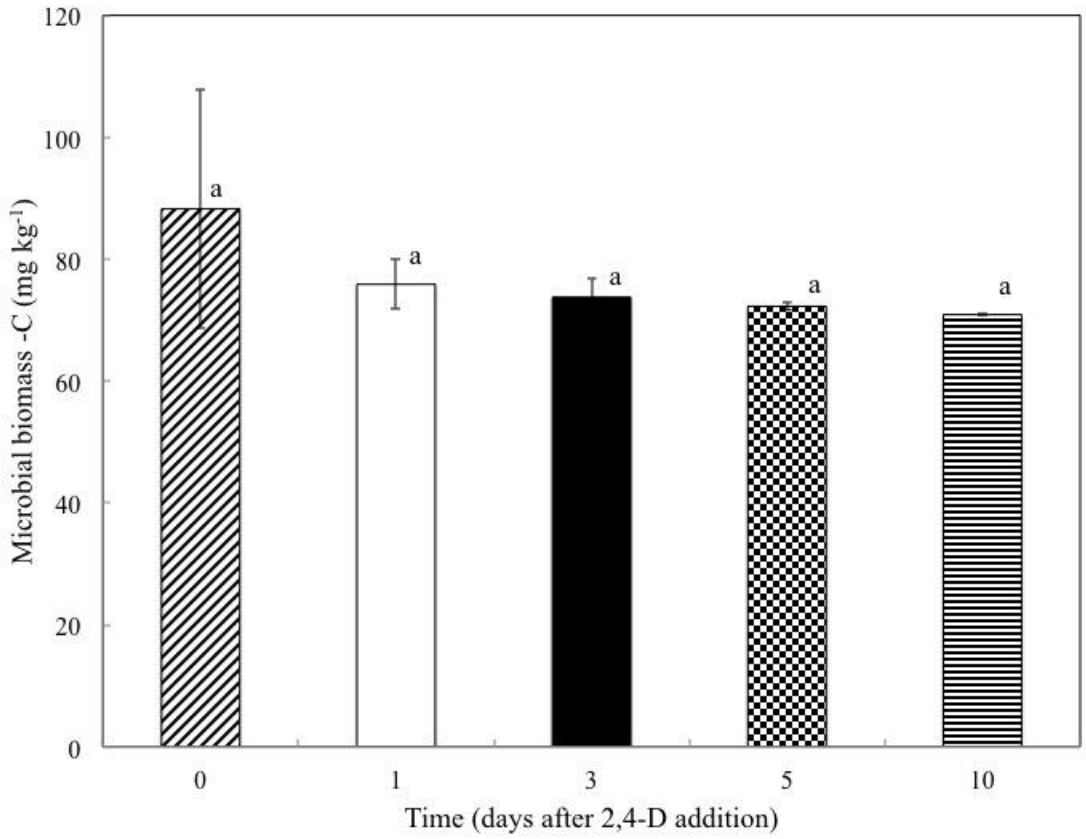

Figure 3.6 Microbial biomass-C estimate from biobed mix incubated at 20 ± 1 ^{o}C for 10 d. Error bars are ± SD of the mean of 3 replicates. Bars with same letters are not significantly different (ANOVA $P \leq 0.05$). The microbial biomass value reported in the control (day zero) represents the mean of two replicates. One replicate was identified as an outlier and was excluded from the calculations.

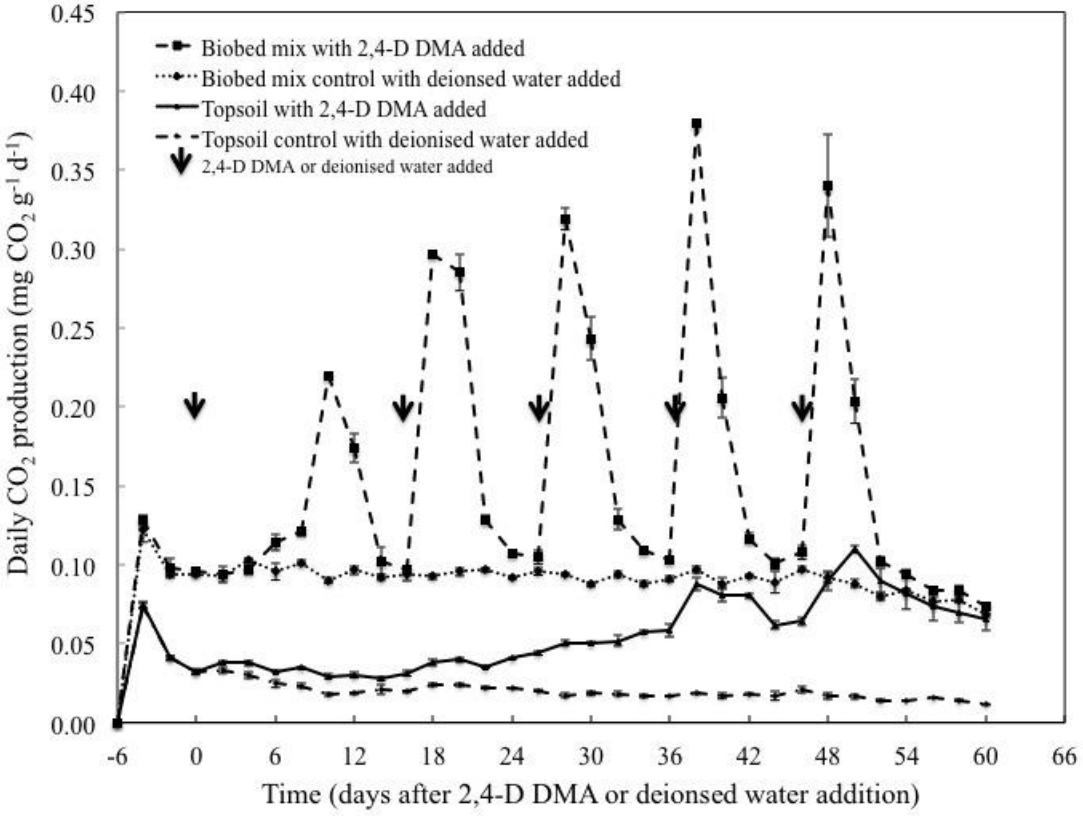

Figure 3.7 Daily CO_2 evolution from the biobed mix to which 2,4-D DMA or deionised water (biobed mix control) was added, and topsoil to which 2,4-D DMA or deionised water (topsoil control) was added over a period of 66 d in the multiple 2,4-D DMA additions experiment. Incubation was at 15 ± 1 ^{o}C. Error bars are ± SD of the mean of 4 replicates and arrows indicate 2,4-D DMA or deionized water addition.

higher (Student's test, $P < 0.0001$) than that produced from topsoil (Figure 3.8).

3.3.2.1 Degradation of 2,4-D DMA in the biobed mix and topsoil multiple additions

The 2,4-dichlorophenoxyacetic acid degraded significantly faster in the biobed mix compared to the topsoil after repeated additions. During 60 days of incubation with repeated 2,4-D DMA additions, 99.7 ± 0.8% and 70.9 ± 1.4% (data not shown) of the applied 2,4-D DMA (259.8 mg a.i kg^{-1}) was degraded in the biobed mix and topsoil, respectively.

3.4 Discussion

3.4.1 Effect of 2,4-D DMA on microbial respiration

Measurement of net CO_2 production is a simple, non-destructive protocol that measures ultimate mineralization and bypasses the theoretical and technical limitations of residue analysis. The burst in CO_2 production noticed during the first 2 d of incubation without 2,4-D DMA was most likely the result of mineralization of both biobed mix and topsoil microbial biomass killed by the drying and wetting process (El-Din Sharabi and Bartha, 1993). Net CO_2 production was used in this study to monitor the microbial activity in the biobed mix and 2,4-D DMA degradation in both the biobed mix and topsoil. For both single and multiple 2,4-D DMA addition experiments, applied 2,4-D DMA-C converted to CO_2 was within the range (≥ 50%), except for the biobed mix in the single 2,4-D DMA addition experiment required for a substance to be classified as biodegradable (Grady, 1985; Strotmann et al., 2004). This showed that 2,4-D DMA was biodegradable in both biobed mix and topsoil used in these experiments. The extent of degradation differed between the two substrates.

In the single 2,4-D DMA addition experiment, 48 and 60% of the added 2,4-D DMA-C was mineralized after 28 d of incubation at 20 ± 1 ^{o}C in the biobed mix and topsoil, respectively. Carbon dioxide production from the biobed mix to which 2,4-D DMA was added lagged for 4 d after 2,4-D DMA addition compared to the control (Figure 3.4). Increase in CO_2 production from the biobed mix control may have been the result of adding water, while addition of 2,4-D DMA could have had a depressing effect on the microorganism. The lag phase was followed by a surge in CO_2 before returning to

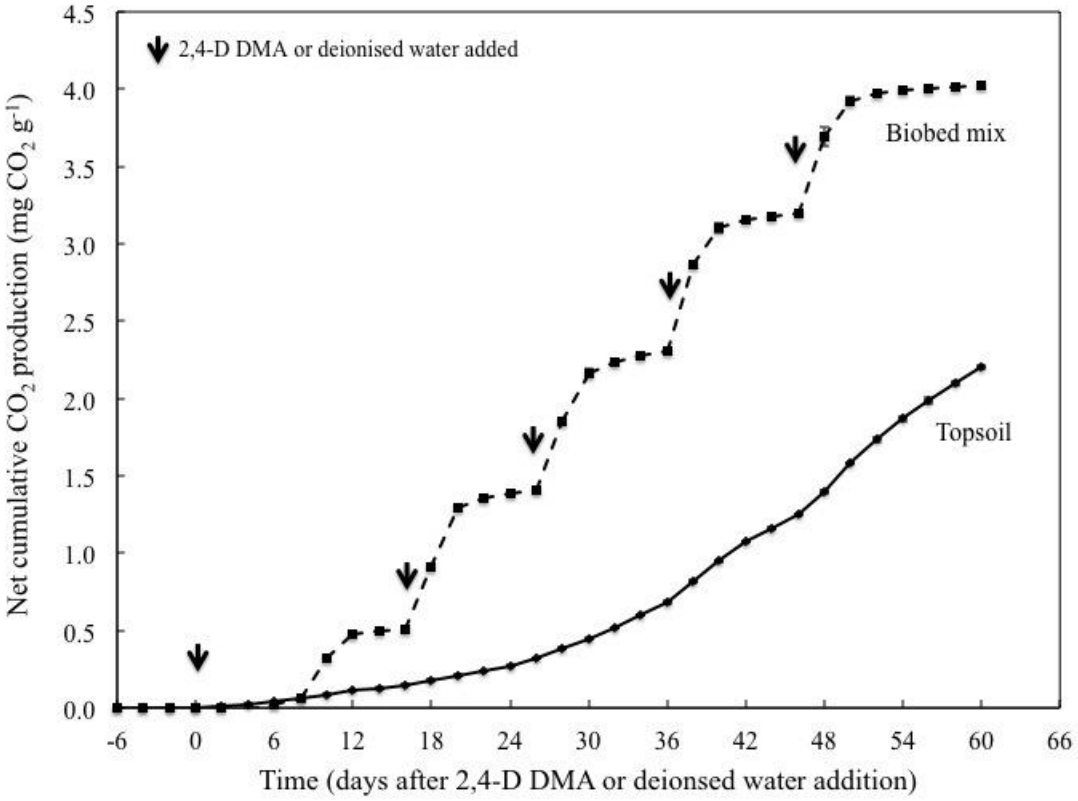

Figure 3.8 Net cumulative CO_2 production (mg CO_2 g^{-1}) from biobed mix and topsoil to which 2,4-D DMA was added (multiple additions) minus their respective controls, incubated at 15 ± 1 ^{o}C. Arrows indicate 2,4-D DMA or deionized water addition.

background levels. The surge in CO_2 production during Phase 2 (i.e. 4 d) represented 52% of 2,4-D mineralization. The presence of lag phase in 2,4-D mineralization in soil has been previously reported and attributed to; 1) enzyme induction, 2) random mutation, and 3) an increase in the number of microorganisms capable of degrading 2,4-D (Estrella et al., 1993; Bousaba et al., 2009). However, the presence or absence of a lag phase in CO_2 production is not well understood. Studies using soil with a history of 2,4-D treatment reported a lag phase of 3 to 4 d (Estrella et al., 1993; Müller et al., 2010). The authors concluded that the lag phase observed in CO_2 production was probably due to an initial small population of 2,4-D degraders that adapted with time. It is not clear if the topsoil used in the preparation of the biobed mix had a history of 2,4-D treatment. Bousaba et al. (2009) reported no lag phase in CO_2 production when two soils with no history of 2,4-D treatment were used. This suggests that the presence or absence of a lag phase after 2,4-D addition into a substrate depends on a variety of factors not only on the population of 2,4-D degraders. One possible factor could be the diversity of the microbial population present in the soil in question, because a variety of soil microorganisms are capable of using 2,4-D as a C source (Prado and Airoldi, 2001). Furthermore, 2,4-D could be toxic to some soil microorganisms and this could lead to a lag phase in its mineralization. There was no noticeable lag phase in CO_2 production in the topsoil samples to which 2,4-D DMA was added compared to the controls. This is in agreement with the finding of Bousaba et al. (2009) who reported no lag phase in two soils with no history of 2,4-D.

The biobed mix MB-C (single 2,4-D DMA addition) showed no significant differences (ANOVA, $P > 0.05$) between the sampling times. The technique used in measuring the MB-C in the biobed mix quantified the entire population rather than just 2,4-D degraders. It is possible that the population of the 2,4-D degraders made up a small proportion of the total microbial population that was measured. Hence, an increase in this subset of the population might not be large enough to be seen in the total microbial biomass. The pattern of the MB-C observed in this study (Figure 3.6) made it impossible to link the CO_2 evolution pattern observed directly to microbial activity. The surge in CO_2 production was not reflected in any significant increases in the MB-C but showed a slight, though insignificant, decrease. The surge in CO_2 may not reflect the degradation of

added 2,4-D DMA alone but also degradation from the mineralization of dead microorganisms. Two studies found a 25 and 60% reduction in MB-C one day after chlorpyrifos at 10 and 50 mg a.i kg^{-1}, respectively, was added to a biobed mix (40% vine straw, 40% green compost, and 20% soil) (Vischetti et al., 2007 and 2008). The authors reported that the microbial population did not recover to pre-application levels by the end of the experiment (62 d). Even with the reduction in MB-C after chlorpyrifos addition, Vischetti et al. (2007) reported half-lives of 43 and 56 d for chlorpyrifos at 10 and 50 mg a.i kg^{-1}, respectively, compared to 84 d at a concentration of 100 mg kg^{-1} in topsoil. In another study, MB-C was negatively influenced when chlorpyrifos and metalaxyl were applied individually or together to the biobed mix made of urban-waste-garden compost, vine branches, and soil (Vischetti et al., 2008). Similar to the current study with 2,4-D DMA, these studies suggest that 2,4-D DMA, chlorpyrifos or metalaxyl degraders constituted a small portion of the total MB.

In the multiple 2,4-D DMA additions experiment, the theoretical amount of C added as a.i. that evolved, as CO_2 was 93 and 51% during 60 days of incubation in the biobed mix and topsoil, respectively. A lag phase in CO_2 production was observed in the biobed mix, as was the case with the single 2,4-D DMA addition experiment. However, subsequent additions to the same substrate produced an immediate release of CO_2. This suggests a build-up of 2,4-D degraders after the initial addition as reported by Macur et al. (2007). In the topsoil, there was a gradual increase in CO_2 produced and a sharp increase was only noticed after the fourth and fifth additions of 2,4-D DMA. This also suggests a slow but gradual build-up of 2,4-D DMA degraders in topsoil over time.

Although there was enhanced 2,4-D DMA degradation in topsoil after repeated additions, the net amount of CO_2 produced was only 51% of the amount of C added. The added C may have been used in the production of new microbial cells or formation of intermediate metabolites (Grady, 1985; Calmon et al., 2000) that could not be degraded by the present microbial population rather than respired as CO_2. It could be possible that not all CO_2 evolved was captured by the CO_2 trap (KOH). However, this study showed that an increase in net CO_2 evolution was related, in time of occurrence and quantity of CO_2 evolved, to 2,4-D DMA degradation in both biobed mix and topsoil.

3.4.2 2,4-D DMA degradation rates

The degradation of 2,4-D and its metabolites under controlled conditions is attributed to the activity of microorganisms, especially fungi and bacteria (Ogle and Warren, 1954; Wardle and Parkinson, 1990; Ismail et al., 2011), which use the compound as a C and energy source (Fournier, 1980; Soulas, 1993; Etinosa et al., 2007). Diverse soil microorganisms are believed to degrade 2,4-D but the diversity, abundance, and activity of 2,4-D degraders vary with SOC, temperature, and moisture conditions (Shymko and Farenhorst, 2008).

The biobed composition is believed to be the main catalyst in the degradation of applied pesticides. Straw stimulates the growth of lignin-degrading fungi and the activity of ligninolytic enzymes (lignin peroxidase, and laccases), which can degrade different pesticides (Torstensson and Castillo, 1997; Von Wirén-Lehr et al., 2001; Spanoghe et al., 2004). Topsoil serves as a source of microorganisms while peat or compost ensures high WHC and a high level of OM for adsorption sites (Spanoghe et al., 2004). Compost also serves a source of microorganisms.

In both single and multiple 2,4-D DMA addition experiments, the biobed mix enhanced 2,4-D degradation compared to topsoil. The rapid degradation observed in the biobed mix could have been as a result of a large microbial population from both topsoil and compost. In the single 2,4-D DMA addition experiment, there was no noticeable 2,4-D degradation within 5 d of 2,4-D DMA addition in the biobed mix. During the same period (5 d), there was a lag in CO_2 production for 3 d. However, 4 d after 2,4-D DMA addition, there was a surge in CO_2 production that peaked on Day 6 and returned to the baseline on Day 10. During this surge (4 d) in CO_2 production, chemical analysis showed that more than 99% of the applied amount of 2,4-D was degraded. This indicates that CO_2 production could be used as an indicator for 2,4-D degradation in the biobed mix.

Slow degradation was observed from the topsoil with 25 and 35% degradation in 10 and 28 d, respectively, after 2,4-D DMA addition in the single 2,4-D DMA addition experiment. Perhaps if the experiment had been continued for a longer period, more 2,4-D degradation could have been observed. The topsoil used in this experiment was stored for more than 2 yr at room temperature. This may have had an effect on the microbial population (decay of the microbial population capable of degrading 2,4-D), because the

method used in soil collection, processing, and storage could influence its integrity. Typically the soil microbial community is the most affected by storage. Storage that negatively influences the soil microbial community can affect the degradation rates of compounds that are degraded biologically (Trabue et al., 2006). Furthermore, the high 2,4-D DMA concentration (512.7 mg a.i kg^{-1}) used and the soil type could have been hindering factors for 2,4-D degradation. Ou et al. (1978) reported the persistence of 2,4-D in soil at a concentration over 50 mg a.i kg^{-1} and 2,4-D DMA concentration used in this experiment was 10.4 times higher. Also the soil used in this experiment could have been a limiting factor for 2,4-D DMA degradation because 2,4-D degradation is inhibited by adsorption on to clay particles (Ogram et al., 1984) and the soil used was 39% clay. Clay soil will provide numerous sites for adsorption that could limit the availability of 2,4-D in solution for microbial attack. However, in the repeated 2,4-D DMA additions experiment, there was increased degradation with more than 70% of applied amount degraded from the topsoil. This result is in agreement with the notion that repeated applications of a pesticide induce the selection of microorganisms capable of utilizing the pesticide as a source of nutrients and energy, thereby enhancing degradation of the parent compound or metabolite (Smith and Mortensen, 1991; Davis et al., 1994). Another possible explanation for why there was high degradation of 2,4-D in the multiple 2,4-D DMA additions experiment compared to single addition experiment from the topsoil could be that after repeated additions, there was an increase (build-up) in the number of 2,4-D degraders, although this was not evident with the quantity of CO_2 that was produced during the incubation period. Maybe the adsorption sites were saturated after a couple of 2,4-D DMA additions leaving substantial amounts of free 2,4-D DMA in solution that might have been used by the enhanced microbial 2,4-D degraders. Generally, there was no difference in 2,4-D degradation in the biobed mix with single or multiple additions because more than 99.9% of applied amount of 2,4-D was degraded in both experiments.

Results obtained in these studies are in agreement with the findings of Fogg et al. (2003b) who also found a significant difference between the degradation of six pesticides (isoproturon, chlorothalonil, pendimethalin, chlorpyrifos, epoxiconazole, and dimethoate) in a single addition to the biobed mix compared to topsoil. Although 2,4-D degradation in biobeds had not been reported in the literature, the results obtained from both single and

multiple 2,4-D DMA additions are in agreement with other pesticides studied so far using the biobed mix as a matrix. For example more than 99% of applied amount of malathion and glyphosate were degraded within six months in a biobed mix (De Roffignac et al., 2008), while more than 98% of applied amount of isoproturon, dimethoate, and mecoprop-P were degraded in a biobed within 12 months of application (Fogg et al., 2004b).

3.5 Conclusion

For both single and multiple additions of 2,4-D DMA, net CO_2 production could be used as an indicator of a.i. breakdown in the biobed mix and topsoil. 2,4-dichlorophenoxyacetic acid degraded faster in the biobed mix than in the topsoil. In the single addition experiment, 99 and 25% of the applied amount of 2,4-D DMA was degraded in the biobed mix and topsoil, respectively, within 10 d of addition.

These results show 2,4-D DMA was degraded faster in the biobed mix than topsoil in both single and multiple additions. The results of MB-C were inconclusive and therefore more research is needed in this area.

4.0 DEGRADATION OF SIX PESTICIDES IN A BIOBED MIX: THE EFFECT OF TEMPERATURE

4.1 Introduction

Over the past half century, widespread use of pesticides has led to their detection in soil, surface water, and groundwater around the world (Kopling et al., 1998; Fava et al., 2005; Hildebrandt et al., 2008). Once pesticides are introduced into the soil environment, their degradation is influenced by factors such as soil composition, nutrient availability, biological properties, and temperature, as well as the chemical composition of the pesticide itself (Walker et al., 2000; Kah et al., 2007). Sorption and degradation are two key processes affecting the transport of pesticides in the soil environment and they are affected by temperature (Sarmah et al., 1998; Soulas and Lagacherie, 2001; Boivin et al., 2005; Si et al., 2005).

The influence of temperature on pesticide sorption can be positive or negative. Temperature typically increases the solubility of pesticides in solution, which could lead to leaching as the pesticides become more mobile (Gupta et al., 2006). On the other hand, increased pesticide solubility will reduce sorption and this could increase degradation as a result of greater partitioning of the pesticide into the liquid phase rendering the pesticide available for microbial attack (Bolan and Baskaran, 1996).

Temperature affects the microbial degradation of pesticides in the soil environment by influencing the rate of microbial growth and activity (Zhang et al., 1993). The activity of microorganisms is stimulated by increases in temperature with a maximum growth rate in soil occurring at 25 to 35 ^{o}C for mesophiles (Mohamed et al., 2011), while thermophiles have higher optimum temperature. High temperature has been associated with increased degradation of some pesticides including 2,4-D, bromoxynil, terbuthylazine, and metamitron (Zhu et al., 2004).

The sulfonylurea herbicides (for example metsulfuron-methyl, thifensulfuron-methyl, and tribenuron-methyl) are a class of chemicals developed in the mid-1970s (Brown, 1990; Sarmah et al., 1998). They are used to control weeds in a variety of crops such as canola, wheat, barley, oat, rice, maize, turf, soybean, oilseed rape, flax, sugar beet, plantation crops, pasture, forestry, blueberry, potato, and tomato (Sarmah and Sabadie, 2002). They have low toxicity to mammals, are highly toxic to plants and are

used at low rates of application (3 to 40 g a.i. ha^{-1}). The most important pathway for their degradation in soil is through chemical hydrolysis and microbial degradation, while other processes such as volatilization and photolysis are relatively insignificant (Fahl et al., 1995). Field and laboratory studies have identified soil pH and OM as the principal factors controlling their mobility and degradation in soil (Si et al., 2005). Metsulfuron-methyl is highly persistent in soil with average half-life under growing conditions ranging between 7 and 42 d (Menne and Berger, 2001). Thifensulfuron-methyl and tribenuron-methyl do not persist in soil for long with average half-lives between 1 and 12 d (Menne and Berger, 2001; Bhattacharjeel and Dureja, 2002).

Thiencarbazone-methyl and pyrasulfotole are two herbicides introduced to Canadian farmers by Bayer CropScience in 2007 and 2008, respectively. They are used to control grass and broadleaf weeds in spring and autumn production in western Canada. Thiencarbazone-methyl is degraded in soil through microbial and chemical processes and the parent molecule and its metabolites do not bind to soil particles. As a result, they have the potential to leach into groundwater or be transported in run-off into surface water. Pyrasulfotole and its main metabolite (pyrasulfotole-benzoic acid) are moderately persistent in soil. It is estimated that 19% of an applied amount is expected to be carried over to the next cropping season (Health Canada 2010). Pyrasulfotole is degraded in soil through microbial and chemical processes (Kaune et al., 2008).

Bromoxynil, a phenolic benzonitrile-based pesticide, is formulated together with pyrasulfotole in the commercial product Infinity. Bromoxynil is used for the control of broadleaf weeds in grain crop production (Chelme-Ayala et al., 2010). Bromoxynil is degraded in soil mainly by microorganisms to form 3,5-dibromo-4-hydroxybenzamide and 3,5-dibromo-4-hydroxybenzoic acid (Golovleva et al., 1988). It has been detected in surface water, soil, and groundwater across the Canadian prairies (Miller et al., 1995; Cessna et al., 1997).

2,4-dichlorophenoxyacetic acid has been widely used across Canada since its introduction more than 60 yr ago. It was the most frequently detected herbicide in groundwater in Canada in the 1990s (Grover et al., 1997). It is used as a post-emergent herbicide in the control of broadleaf weeds in cereal crops, turf, pastures, and non-crop lands (Cullimore, 1981). 2,4-dichlorophenoxyacetic acid is degraded in soil primarily

through microbial degradation (Wilson et al., 1997) to form 2,4-dichlorophenol (Gonod et al., 2006). The adsorption of 2,4-D to humus and clay minerals is low due to its high polarity and negative charge when present as the carboxylate; as a result, it has a high potential to leach into groundwater (Felding, 1995; WenHai et al., 2009).

Since the introduction of biobeds in Sweden in 1993, various field and laboratory studies have been conducted to test their performance. These studies tested a wide range of pesticides and various raw materials and concluded that biobeds were efficient in degrading the studied pesticides (Fogg et al., 2004b; Castillo and Torstensson, 2007; De Wilde et al., 2010). For example the half-lives of terbuthylazine, dimethoate, metalaxyl-M, metribuzin, buprofezin, iprodione, indoxacarb, and azoxystrobin in the biobed mix were 7.8, 5.8, 34.7, 21.0, 28.9, 31.5, 53.3, and 25.7 compared to 99.0, 5.9, 173.3, 34.7, 86.6, 86.6, 231.0, and 69.3 d in soil, respectively (Karanasios et al., 2010a). Some pesticides with use registered in Canada that have been studied in the biobed include metsulfuron-methyl, bromoxynil, chlorpyrifos, azoxystrobin, bentazon, dimethoate, diuron, and glyphosate. For biobeds to be useful in the Canadian prairies, they must be able to degrade a variety of pesticides. Temperature is a major factor that determines the microbial activity of any biological system such as the biobed. Saskatchewan in particular and the prairies in general are known for extremely cold winters and warm short summers. These temperatures could be a major factor affecting the performance of biobeds in this part of the world. So far, only Castillo and Tornstensson (2007) have investigated the effect of temperature on pesticide degradation in a biobed mix, and reported higher degradation rates at higher temperatures for most of the studied pesticides. The present study will extend the existing knowledge on the performance of biobeds on the degradation of seven pesticides (with contrasting physiochemical properties) commonly used in the Canadian prairies at various temperatures.

4.1.2 Objective

The objective of this study was to study the effect of temperature on the degradation of seven pesticides with contrasting physiochemical properties in a biobed mix.

4.2 Materials and Methods

4.2.1 Preparation of biobed mix

The biobed mix was prepared by mixing topsoil, composted cattle manure, and chopped wheat straw in a ratio of 1:1:2 (v/v/v). For a complete description of the biobed mix preparation and chemical properties of the biobed mix, compost, topsoil, and straw see section 3.2.2.

4.2.2 Test chemicals

The seven commercially formulated pesticides used in this study and their physicochemical properties are presented in Table 4.1. Metsulfuron-methyl (methyl 2-[[[[4-methoxy-6-methyl-1,3,5-triazinyl) amino] carbony] amino] sulfony] benzoate, tribenuron-methyl (methyl 2-[[[[N-(4-methoxy-6-methyl-1,3,5-triazinyl)methylamino]carbonyl]amino]carbonyl]amino]sulfonyl] benzoate and thifensulfuron-methyl (methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbony]amino]sulfony]-2-thiophenecarboxylate were purchased from DuPont chemical company (Saskatoon, SK). Thiencarbazone-methyl (methyl 4-[4,5-dihydro-3-methoxy-4-methyl-5-oxo-1*H*-1,2,4-triazol-1-yl)carboxamidosulfonyl]-5-methylthiophene-3-carboxylate, pyrasulfotole (5-hydroxyl-1,3-dimethyl-1*H*-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl) phenyl] methanone; bromoxynil (3,5-dibromo-4-hydroxybenzonitrile), and 2,4-D dimethylamine salt were donated by Bayer CropScience (Regina, SK).

4.2.3 Criteria used for the selection of pesticides

Metsulfuron-methyl, tribenuron-methyl, and thifensulfuron-methyl were included in this study due to their average annual usage in the Canadian prairies, their detection in surface water and groundwater (Cessna et al., 2006; Donald et al., 2007), persistence in alkaline soils, mode of degradation (Cranmer et al., 1999), their solubility in water, and high mobility in soils (Fahl et al., 1995).

Thiencarbazone-methyl and pyrasulfotole were selected on the basis that no extensive degradation studies have been carried out regarding their mobility and/or

Table 4.1 Studied pesticides and their reported physicochemical characteristics.

Active ingredient	Product name	Conc. (g L^{-1})	Mobility	$T_{1/2}$ (d)[†]	Water solubility (mg L^{-1})	K_{oc} (mL g^{-1})[‡]
Metsulfuron-methyl[§]	Ally	600	Very Mobile	11-38	2790	4-206
Tribenuron-methyl[¶]	Express	500	High	1-10	2040	30-80
Thifensulfuron-methyl[¶]	Pinnacle	500	Very high	2-6	2240	13-55
Thiencarbazone-methyl[#]	Velocity	10	High	3.2-55	436	59.9-236
Pyrasulfotole[#]	Infinity	37.5	Moderate	6-18	69100	21.5-715
Bromoxynil[#]	Infinity	210	Moderate	7	130	202
2,4-D[¶]	2,4-D	560	High	7	27600	46

[†]$T_{1/2}$ = Reported half-life in soil.
[‡]K_{oc} = Adsorption coefficient; a high value indicates less mobility.
[§]= Agricultureal Research Service (ARS), 2010.
[¶]= Cessna et al. (2010).
[#]= EPA, 2007.

persistence in a biobed mix.

Bromoxynil is used in the control of broadleaf weeds in cereal production in the Canadian prairies and has been found in the water of 50 to 71% of dugouts sampled across Saskatchewan (Grover et al., 1997).

2,4-dichlorophenoxyacetic acid is widely used as a post-emergence herbicide to control weeds in cereal production and has been found in groundwater across Canada (Waite et al., 1992 and 2005). It has a relatively short persistence and low affinity in soil (Guo et al., 2000).

4.2.4 Calculation of pesticide concentration

The choice of application amount of each pesticide was based on a hypothetical scenario of a 3900-L sprayer tank that contained label-recommended rates of the various pesticides for field application. It was assumed that 1% (39 L) of tank charge was left in the tank after draining and the wash volume after spraying was estimated to be 10% of tank charge (recommended volume). Therefore the amount of a.i. of each pesticide left in the spray after field application and washing was calculated as follows: = (Volume of liquid left after spraying) / (Wash volume + volume of liquid left after spraying)*100% = $\%(39L)/(390L+39L)*100$, equivalent to 9% of original 1% tank charge concentration.

For estimating pesticide concentration per unit mass of biobed mix, the following assumptions were made: 1) pesticides were limited to the upper 10 cm of the biobed mix and were uniformly spread over the biobed area of 9 m^2, 2) pesticide concentration in the sprayer was equivalent to the field recommended rates, 3) detection of 1% of the applied amount assuming 99% degradation, and 4) LC/MS/MS detection threshold of 1 ng mL^{-1}.

2,4-D dimethylamine salt is used as an example of how each pesticide amount used was calculated. 2,4-D dimethylamine salt has a product application rate of 400 g ha^{-1} (equivalent to 560 g a.i. ha^{-1}) applied in a 100-L application volume (100 L ha^{-1}). The number of hectares covered per sprayer was obtained by dividing the sprayer tank volume by the application volume $(3900\,L/100\,L\,ha^{-1}=39\,ha)$. The amount of 2,4-D DMA per full tank (recommended amount) was calculated as 2,4-D DMA a.i. in the formulated product multiplied by the number of hectares covered by a sprayer with a full tank $(560\,g\,a.i.\,ha^{-1}*39\,ha=21840\,g\,a.i.)$. The tank charge per sprayer was calculated as

the amount of 2,4-D DMA in a full tank divided by the sprayer volume ($21840\ g\ a.i. / 3900\ L = 5.6\ g\ a.i.\ L^{-1}$). The amount of 2,4-D DMA left in the tank after the spray operation was obtained by multiplying the amount of liquid left in the tank by the concentration of 2,4-D DMA ($39\ L * 5.6\ g\ a.i.\ L^{-1} = 218.4\ g\ a.i.$). The concentration of 2,4-D DMA a.i. left in tank wash was then calculated by dividing the amount of 2,4-D DMA left in the sprayer tank by the sum of the wash volume plus leftover liquid in the tank $\left(218.4\ g\ a.i. / (390 + 39)\ L\right) = 0.51\ g\ a.i.\ L^{-1}$. Therefore the total amount of 2,4-D DMA a.i. in the upper 10 cm of the biobed (4933 kg) material was calculated as the amount of 2,4-D DMA left in the tank after the spray operation divided by biobed dry weight. In the province of Saskatchewan, there are usually five pesticide applications in a typical growing season, which will result in five cleanouts. Hence the biobed is expected to receive a total of five times the concentration of one cleanout ($5 * 0.024\ g\ a.i.\ kg^{-1} = 0.12\ g\ a.i.\ kg^{-1}$).

4.2.5 Experimental setup

4.2.5.1 Incubation temperature

Field application of pesticides in Saskatchewan usually starts in early May. To select the field biobed temperature for this May, average daily soil temperature data (0 to 100-cm depth) were obtained from May 1st to 15th from 1999 to 2008 from the University of Saskatchewan's Kernen Crop Research Farm (www.usask.ca/weather/kfarm/data/?C=M;O=D). The calculated average daily temperature was 6.7 ^{o}C (0 to 100-cm depth). However, due to differences in composition between the biobed mix and soil, 5 ^{o}C was selected as the starting temperature. The other temperatures (13 and 20 ^{o}C) used in this study were arrived at after calculating the monthly soil temperature from 0 to 100 cm for the years 2005, 2007, and 2008 (Appendix A). These temperatures (5, 13 and 20 ^{o}C) fall within the temperature range a Saskatchewan field biobed is expected to experience within a typical growing season from early May to late September (Table A-2).

4.2.5.2 Incubation apparatus

A thermo-gradient plate (Figure 4.1) was used for the incubation. A thermo-

Figure 4.1 A thermo-gradient plate apparatus used for the incubation of biobed mix at three temperatures (5, 13, and 20 ^{o}C).

gradient plate is an instrument with precise temperature control that maintains a constant temperature over a specific period of time. The temperature within each one of the 80 cells is independently controlled and recorded automatically. The basic unit is composed of an aluminum bar insulated on three sides with expanded polystyrene, heated electrically at one end and cooled at the other with a semi-conductor heat pump. The temperature from one end of the bar to the other is determined by the balance between heat input and removal, controlled separately at the hot and cold ends of the bar (Fox and Thompson, 1971).

The experiment was established as a randomized complete block design with 4 replicates and two factors (incubation temperature and sampling time). Air-dried biobed mix was removed from storage (4 ± 1 ^{o}C) and partially re-hydrated in a cement mixer. The re-hydrated biobed mix was allowed to equilibrate at room temperature for 1 wk with periodic turning. Twenty-five grams odw of partially re-hydrated biobed mix was weighed and placed into Petri dishes. The required moisture content of 70% (w/w) WHC was achieved by addition of deionised water to the biobed mix in the Petri dishes.

Individual solutions with 150 mg a.i. L^{-1} of each of the pesticides except bromoxynil were prepared in deionised water. The following amounts of formulated pesticide product were added in 50-mL deionised water: 0.12 g metsulfuron-methyl (Ally, 60% w/v, 2,400 mg L^{-1}), 0.15 g tribenuron-methyl (Express, 50% w/v, 3,000 mg L^{-1}), 0.15 g thifensulfuron-methyl (Pinnacle, 50% w/v, 3,000 mg L^{-1}), 7.5 mL of thiencarbazone-methyl (Velocity, 10% w/v, 75 mg L^{-1}). Pyrasulfotole and bromoxynil were formulated together (and called Infinity). Two mL of Infinity containing 37.5 g L^{-1} pyrasulfotole equivalent of 75 mg L^{-1} and 210 g L^{-1} bromoxynil equivalent of 420 mg L^{-1} product was added to 50-mL deionised water. For the 2,4-D DMA, 0.27 mL of 2,4-D DMA (2,4-D DMA, 560% w/v, 151.2 mg L^{-1}) was mixed in 100 mL of deionised water.

A dilute stock solution of a mixture of the pesticides was prepared by mixing 10 mL of each pesticide solution into a 100-mL flask to a final volume of 100 mL. This resulted in final individual concentrations of 1.5 mg a.i. L^{-1} of each of the pesticides. One mL of the diluted stock solution containing 1.5 mg a.i. L^{-1} of metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, 2,4-D DMA, respectively, and 8.4 mg a.i. L^{-1} bromoxynil was applied to each replicate of the

biobed mix in the Petri dishes. The concentration of bromoxynil used was higher than the rest of the pesticides because it was formulated together with pyrasulfotole that has a low field application rate (Table 4.1). The pesticide solution was distributed over the biobed mix in the Petri dishes using a pipette. One mL of deionised water was applied to the controls at the same time. Petri dishes were incubated at 5, 13, and 20 ± 0.2 ^{o}C in the dark. Every second day, the Petri dishes were removed from the incubator and weighed. Any loss in moisture was adjusted to 70% WHC by the addition of deionised water (Vischetti et al., 2008). The experiment was carried out once and produced a total of 68 samples (3 temperatures x 4 replicates x 5 sampling times + 4 replicates without pesticides, and 4 replicates with pesticides and immediately placed in a freezer for recovery calculations at -20 ± 2 ^{o}C for a time zero measurement).

4.2.5.3 Sampling for pesticide residues

Sampling was carried out weekly. At each sampling time, 4 replicates of treated biobed mix at each temperature under investigation were removed from the incubator. The sampled material was placed into plastic bags, sealed and immediately stored at -20 ± 2 ^{o}C. Eight samples at time zero (4 replicates with pesticides and 4 replicates without pesticides) were immediately placed in a freezer at -20 ± 2 ^{o}C in sealed plastic bags (Brown et al., 1997).

4.2.6 Pesticide analyses

4.2.6.1 Extraction

At the end of the experiment samples were removed from storage (-20 ± 2 ^{o}C) and allowed to thaw at room temperature. After thawing, the contents of each Petri dish were transferred into 125-mL flat bottom glass bottles. One hundred mL of extraction solvent (deionised water:acetonitrile, 21:79 v/v) was added and the mixture shaken for 1 h with a front-end Burrell shaker at 50 rpm. The mixture was filtered through a 0.45 µm Whatman^{TM} filter paper (110 mm i.d.) using a Buchner funnel under suction. The final volume of the filtrate was adjusted to 100 mL with additional extraction solvent. The filtrate was thoroughly mixed and a 20-mL aliquot transferred into a vial and maintained at 4 ± 1 ^{o}C prior to chemical analysis.

4.2.6.2 Chemical analysis for metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, and pyrasulfotole

Prior to chemical analysis, 100 µL of extract was diluted in 900-µL deionised water and 20 µL of the aqueous solution injected into a Waters 2695 Alliance Liquid Chromatography-Tandem Mass Spectrometer (LC-MS/MS). Herbicide residue analysis was achieved through a two-step process.

Step 1: Separation of pesticides under investigation:

Pesticides under study were separated using a Waters 2695 Alliance High Performance Liquid Chromatography (HPLC) system (Water Ltd. Mississauga, ON) with a Waters Xterra Mass C_{18} (2.1 mm x 100 mm internal diameter, 3.5µm particle size diameter) (Water Ltd. Mississauga, ON). The analytical column was maintained at room temperature. The mobile phase consisted of solvent A (90:10 water: acetonitrile) and solvent B (90:10 acetonitrile: water). Both solvents contained 0.1% (v/v) formic acid and 2 mM ammonium acetate (Donald et al., 2007). The isocratic elution of the column was with 50% of both solvents (A and B) at a flow rate of 200 µL min^{-1}. This resulted in retention times of 3.81, 5.82, 6.50, 7.22, and 12.88 min for pyrasulfotole, thifensulfuron-methyl, for metsulfuron-methyl, thiencarbazone-methyl, and tribenuron-methyl, respectively.

Step 2: Quantification of pesticides:

Pesticides were quantified and their presence confirmed using the Water Micromass Quattro Ultima triple quadrupole mass spectrometer (Waters Ltd.) equipped with an electrospray ionization (ESI) interface set to positive ion mode. Ionization conditions were optimized by infusing a 0.5 mg L^{-1} solution of each herbicide into the ion source in a 50:50 acetonitrile: water solution with a syringe pump. The masses of parent molecule (M) plus that of hydrogen (H) $(M+H)^+$ for each analyte was selected for fragmentation using the first quadrupole. The second quadrupole, into which argon gas was introduced, functioned as a collision cell and the third quadrupole was used to monitor the resulting major fragment ion (Donald et al., 2007). Suitable multiple reaction monitoring (MRM) or selected reaction monitoring (SRM) transitions were chosen from the product ion scans and were as follows: metsulfuron-methyl [382.3 to 167.2 atomic mass units (amu)], tribenuron-methyl (396.3 to 155.2 amu), thifensulfuron-methyl (388.3

to 167.2 amu), thiencarbazone (391.0 to 359.0 amu), and pyrasulfotole (363.0 to 251.0 amu).

Instrumental conditions were as follows: source temperature, 90 ^{o}C; capillary voltage, 4.39 kV; hex 1 voltage, 6.9 V; hex 2 and aperture voltage, V; desolvation temperature, 220 °C; nitrogen desolvation gas flow rate, 488-L h^{-1}; nitrogen cone gas flow rate, 145-L h^{-1}; nitrogen nebulizer gas flow rate was at maximum flow; multiplier voltage, 650 V; and the interchannel delay was 0.10 s while dwell time ranged from 0.20 to 0.50 s for the three time dependent MRM channels. Argon was used as the collision gas at a pressure, which increased the Pirani gauge reading to 3.53 x 10^{-4} torr. The cone voltage (11 to 16 V) and collision energy (19 to 26 eV) were dependent on the MRM channel. Resolution was set to achieve unit mass resolution for quadrupole 1 and approximately 2 amu resolutions for quadrupole 3 (Cessna et al., 2006). The percentage recovery from the biobed mix (n=4) was 64 ± 0.7, 78 ± 2.8, 81 ± 1.0, 83 ± 0.5, and 85 ± 1.3 for thiencarbazone-methyl, pyrasulfotole, thifensulfuron-methyl, tribenuron-methyl, and metsulfuron-methyl, respectively.

4.2.6.3 Chemical analysis for 2,4-D

2,4-dichlorophenoxyacetic acid was analysed as described in section 3.2.8.2.

4.2.6.4 Chemical analysis for bromoxynil

The following reagents were used for the chemical analysis. High performance liquid chromatography (HPLC) grade acetonitrile was purchased from Caledon Laboratories Ltd. (Edmonton, AB), bromoxynil analytical standard was purchased from ChemService Inc. (West Chester, PA), and formic acid from Sigma Aldrich Canada Ltd. (Oakville, ON). LC Instrument: Waters 2695 Alliance HPLC System.

A Waters 2695 Alliance HPLC system with a Waters Xterra Mass C_{18} was used for bromoxynil analysis. The mobile phase consisted of solvent A (90:10 water:acetonitrile) and solvent B (90:10 acetonitrile:water) both solvents contained 0.1% (v/v) formic acid. Isocratic elution of the column with 50% solvent A and 50% solvent B at a flow rate of 200 µL min^{-1} resulted in bromoxynil retention time of 4.70 min. The injection volume was 20 µL. Bromoxynil residue was quantified using Micromass Quattro Ultima triple quadrupole mass spectrometery equipped with an electrospray

ionization (ESI) interface set to negative ion mode. Ionization was optimized by infusing a 0.5 mg L^{-1} solution of bromoxynil into the ion source in a 50:50 acetonitrile:water solution with a syringe pump. Each sample and calibration standard was treated with bromoxynil internal standard to compensate for variability in the ESI process. The first quadrupole of the mass spectrometer (operating in negative ESI mode) was set to allow the parent ion through. The parent ion [273.7 atomic mass unit (amu)] was transferred to the second stage of the mass spectrometer where collision with helium resulted in fragmentation of the parent ion. The fragment ion (160.8 amu) was allowed to pass through to the third quadrupole of the mass spectrometer where the resulting ions were counted and quantified by comparison to the response given by a calibration curve made-up of a set of 4 analytical standards; 10, 50, 100, and 500 µg L^{-1}, respectively. The percentage recovery from the biobed mix (n=4) was 89 ± 1.8.

4.2.7 Statistical and data analyses

The degradation data (amount of pesticide recovered after incubation) were subjected to two-way analysis of variance (ANOVA) using the CoHort 6.4 software (CoHort 6.4 software, Monterey, CA, 93940, USA). The two main factors (temperature and sampling time) and their interaction were tested for significance ($P \leq 0.05$). The amount of each pesticide remaining after each sampling point was fitted to the FOK Eq. [4.1]. The FOK was used to describe the degradation pattern of the various pesticides. The FOCUS workgroup guidance was used to select the best model that described the degradation results obtained (Si et al., 2005; FOCUS, 2006).

$$\ln C_t - \ln C_0 = -kt \qquad [4.1]$$

Where C_t is the concentration (mg kg^{-1} biobed mix) of each pesticide at time t, C_o is the initial (theoretical) concentration of each pesticide (mg kg^{-1} biobed mix), t is the time (d), and *k* the degradation rate constant (d^{-1}). A plot of *ln* (C_t/C_o) versus time yielded a straight line with slope equal to *k*. The degradation rate constant (*k*) was then used to calculate the half-life ($t_{1/2}$), the time at which the applied amount reached half the initial amount Eq. [4.2].

$$t_{1/2} = \ln 2 / k \qquad [4.2]$$

When data showed increased degradation with time, time for 90% (t_{90}) of the applied amount to be degraded was calculated using Eq. [4.3].

$$t_{90} = \ln 10 / k \qquad [4.3]$$

When the amount of each pesticide remaining at the end of each sampling point was below the limit of detection (1 ng mL^{-1}), 0.5 ng mL^{-1} was used as the amount remaining. The Arrhenius equation Eq. [4.4] was used to study the effect of temperature on pesticide degradation

$$k = Ae^{-E_a/RT} \qquad [4.4]$$

Where k is the degradation rate constant, E_a is the activation energy of the reaction (J mol^{-1}), R is the gas constant (JK^{-1} mol^{-1}), T is the absolute temperature (Kelvin) and A is the empirical constant (d^{-1}). Taking the natural logarithm (*ln*) of both sides of Eq. [4.4] gives Eq. [4.5]

$$ln\, k = -Ea / RT + \ln A \qquad [4.5]$$

A plot of *ln k* against 1/T gives a straight line with a slope of $– E_a/R$. A high activation energy (E_a) means the reaction is more sensitive to temperature.

The temperature coefficient (Q_{10}) shows the factor by which the rate (k) of the reaction will increase with every 10-degree rise in temperature Eq. [4.6] was also employed to study the effect of temperature on pesticide degradation.

$$Q_{10} = \left(k_2 / k_1\right)^{\{10/(T_2 - T_1)\}} \qquad [4.6]$$

Where Q_{10} is the factor by which the reaction rate increases when the temperature (T) is raised by 10-degree, k_1 *and* k_2 are degradation rate constants, at T_1 and T_2, respectively.

4.3 Results

4.3.1 Effect of temperature on pesticide degradation

The amount of pesticide remaining in the biobed mix was a function of time elapsed and temperature (with less remaining as time and temperature increased). The pesticides differed in their specific response to time, temperature, and the interaction between time and temperature.

The amount of metsulfuron-methyl remaining in the biobed mix was a function of both temperature and time with no significant interaction (Table 4.2). The degradation of metsulfuron-methyl in the biobed mix at all three temperatures was very low with 29, 33, 38% of the applied amount (6 g kg^{-1}) degraded within 35 d at 5, 13, and 20 ^{o}C, respectively (Figure 4.2a).

The amount of tribenuron-methyl remaining in the biobed mix was a function of both temperature and time with no significant interaction (Table 4.2). The degradation of tribenuron-methyl in the biobed mix at all three temperatures was higher than that of metsulfuron-methyl with 52, 73, and 94% of the applied amount (6 g kg^{-1}) degraded within 35 d at 5, 13, and 20 ^{o}C, respectively (Figure 4.2b).

The amount of thifensulfuron-methyl remaining in the biobed mix was a function of temperature and time, and the time response depended on the temperature (Table 4.2). The degradation of thifensulfuron-methyl in the biobed mix at all three temperatures was higher than that of tribenuron-methyl with 97, 97, and 98% of the applied amount (6 g kg^{-1}) degraded within 35, 21, and 14 d at 5, 13, and 20 ^{o}C, respectively (Figure 4.2c).

The amount of thiencarbazone-methyl remaining in the biobed mix was a function of both temperature and time with no significant interaction (Table 4.2). The degradation of thiencarbazone-methyl in the biobed mix at all temperatures was higher than that of metsulfuron-methyl but lower than that of tribenuron-methyl with 65, 70, and 77% of the applied amount (6 g kg^{-1}) degraded within 35 d at 5, 13, and 20 ^{o}C, respectively (Figure 4.2d).

The amount of pyrasulfotole remaining in the biobed mix was a function of temperature and time, and the time response depended on the temperature (Table 4.2). The degradation of pyrasulfotole in the biobed mix at all three temperatures was less than

Table 4.2 ANOVA table showing the effects of time, temperature and their interaction on the degradation of metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, bromoxynil, and 2,4-D in a biobed mix (pH 7.4) incubated at three temperatures (5, 13, and 20 ^{o}C) over a period of 35 d.

		Metsulfuron-methyl			Tribenuron-methyl			Thifensulfuron-methyl			Thiencarbazone-methyl		
Source of variance	df[†]	MS[‡]	F	*P*	MS	F	*P*	MS	F	*P*	MS	F	*P*
Temperature	2	63919	43	0.001	1970018	319	0.001	101651	1966	0.001	97364	201	0.001
Time	4	91640	62	0.001	456191	75	0.001	91027	1761	0.001	74174	153	0.001
Interaction	8	722	1	0.857	2963	1	0.846	11184	216	0.001	880	2	0.099
Error	45	1475			6171			52			485		

		Pyrasulfotole			Bromoxynil			2,4-D		
Source of variance	df	MS	F	*P*	MS	F	*P*	MS	F	*P*
Temperature	2	491347	395	0.001	24839220	1697	0.001	992144	1265	0.001
Time	4	253078	204	0.001	12351937	844	0.001	344149	439	0.001
Interaction	8	32665	26	0.001	967280	66	0.001	245692	313	0.001
Error	45	1243			14635			784		

[†]df = degree of freedom.
[‡]MS = mean square.
F= F statistic from ANOVA.
P= probability level that the F statistic and hence the source of variance is significant.

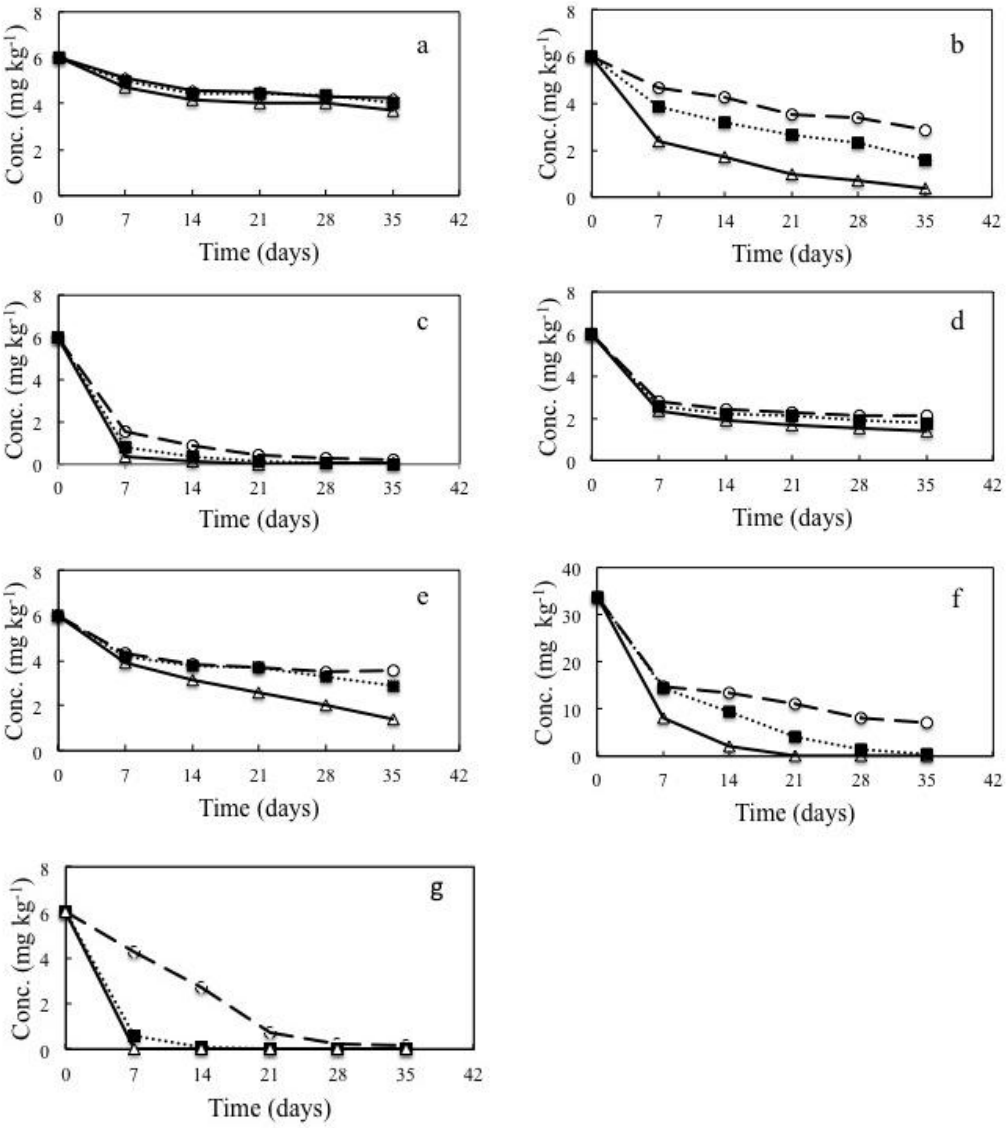

Figure 4.2 Degradation of (a) metsulfuron-methyl, (b) tribenuron-methyl, (c) thifensulfuron-methyl, (d) thiencarbazone-methyl, (e) pyrasulfotole, (f) bromoxynil, and (g) 2,4-D over time incubated at 5 (O), 13, (■), and 20 (Δ) ^{o}C, in a thermo-gradient plate for 35 d.

that of thiencarbazone-methyl with 41, 52, and 77% of applied amount (6 g kg^{-1}) degraded within 35 d at 5, 13, and 20 ^{o}C, respectively (Figure 4.2e).

The amount of bromoxynil remaining in the biobed mix was a function of temperature and time, and the time response depended on the temperature (Table 4.2). The degradation of bromoxynil in the biobed mix at all three temperatures was higher than all studied pesticides with the exception of thifensulfuron-methyl and 2,4-D, with 79, 96, and 94% of the applied amount (33.6 g kg^{-1}) degraded within 35, 28, and 14 d at 5, 13 and 20 ^{o}C, respectively (Figure 4.2f).

The amount of 2,4-D DMA remaining in the biobed mix was a function of temperature and time, and the time response depended on the temperature (Table 4.2). The degradation of 2,4-D DMA in the biobed mix at all three temperatures was higher than all other six pesticides (metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and bromoxynil) with more than 99% of applied amount (6 g kg^{-1}) degraded within 35, 14, and 7 d at 5, 13, and 20 ^{o}C, respectively (Figure 4.2g).

For each temperature, linear regressions were calculated. The degradation rate constants (k) were determined as the slope of the regression line. The theoretical initial amount at time zero was used to calculate the amount remaining at each sampling period. However, pesticide recovery rates from the biobed mix were not taken into account in the calculations. The degradation rate constants were used to calculate half-life (ln 0.5/k) and time for 90% of the applied amount to degrade (ln 10/k), except for metsulfuron-methyl at all temperatures and pyrasulfotole at 5 ^{o}C because degradation was less than 50% (Table 4.3).

The degradation of pesticides in soil is the result of a combination of chemical and biological factors. The temperature dependence of the degradation rate can be described by determining the activation energy of the reaction using the Arrhenius equation (Ismail et al., 2011). To better, characterize the effect of temperature on pesticide degradation in this study, the Arrhenius equation expressed as the natural logarithm (ln) was employed [Eq. 4.5]. The activation energy indicates the sensitivity of the reaction to temperature changes. The higher the activation energy, the greater the increase in the reaction rate with a given increase in temperature (Starner et al., 1999;

Bondarenko et al., 2004). Estimated activation energies (E_a), of the individual pesticides (Table 4.4; Figure 4.3) were calculated by multiplying the slope of the linear regression by the universal gas constant R (8.31) (Dinelli et al., 1997; Saha and Kulshrestha, 2008). Calculations were not performed for 2,4-D because the degradation was so rapid at 20 and 13 ^{o}C that after one and two weeks, respectively, the amount remaining was below the limit of detection. For metsulfuron-methyl, degradation was too slow at all temperatures for the activation energy to be estimated. The similar activation energies of tribenuron-methyl, thifensulfuron-methyl, and bromoxynil, (61.22, 64.19, and 61.19 kJ mol^{-1}, respectively) indicate a similar degradation pattern as a function of temperature. The activation energy of thiencarbazone-methyl (26.49 kJ mol^{-1}) was lower than the rest of the pesticides and indicates a different degradation pattern. The activation energy for pyrasulfotole was calculated using 13 and 20 ^{o}C because the degradation at 5 ^{o}C was below 50% of the applied amount (6 g kg^{-1}). The calculated activation energy for pyrasulfotole was 105.55 kJ mol^{-1} indicating a different rate of degradation.

4.4 Discussion

The extent to which pesticides are retained and degraded within the biobed determines its performance. For the biobed mix to be efficient, it must promote pesticide binding, develop an efficient and robust microbial flora with durable pesticide degradation capacity capable of tolerating pesticides applied to it at high concentration in a mixture or individually (Castillo et al., 2008). Compost enhances the sorption capacity of the biobed mix and provides microorganisms, topsoil provides microorganisms, and straw promotes the proliferation of microorganisms with the ability to degrade pesticides characterized by an aromatic ring structure (Spliid et al., 2006).

The highest rate of degradation of all pesticides in this study was at 20 ^{o}C except for metsulfuron-methyl, which showed little response to temperature. The result obtained in this study for the seven pesticides are in agreement with those reported by Castillo and Torstensson (2007). These authors reported a higher degradation rate for metamitron, metribuzin, isoproturon, terbuthylazine, chloridazon, methabenzthiazuron, and linuron at 20 ^{o}C in a biobed mix compared to 2 and 10 ^{o}C, respectively.

The various rates of degradation observed in this study (Figure 4.2a-g) suggest that there might be different degradation pathways even with pesticides from the same

Table 4.3 Temperature, $T_{1/2}$ and T_{90}, degradation rate constants (*k*), and coefficients of determination (r^2) for tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, bromoxynil, and 2,4-D incubated at three temperatures (5, 13, and 20 ^oC) over a period of 35 d.

Pesticides	Temperature	$T_{1/2}$ (d)[†]	T_{90} (d)[‡]	*k* (d^{-1})[§]	r^2
Tribenuron-	5	40.5	134.7	0.017	0.979
methyl	13	23.5	78.1	0.030	0.971
	20	10.3	34.3	0.067	0.979
Thifensulfuron-	5	9.1	30.1	0.077	0.979
methyl	13	4.7	157.0	0.147	0.994
	20	2.2	7.2	0.319	0.935
Thiencarbazone-	5	70.1	232.6	0.009	0.912
methyl	13	55.9	185.7	0.012	0.982
	20	38.7	128.6	0.018	0.963
Pyrasulfotole	5[¶]				
	13	56.4	187.2	0.012	0.954
	20	19.5	64.9	0.036	0.986
Bromoxynil	5	40.5	134.7	0.017	0.979
	13	23.5	78.1	0.029	0.971
	20	10.3	34.3	0.067	0.979
2,4-D	5	5.2	17.2	0.1306	0.964
	13	2.3	7.5	0.306	1.000
	20[#]				

[†]$T_{1/2}$ = Calculated half-life in biobed mix.
[‡]T_{90} = Time for 90% of applied a.i. to degrade.
[§]*k* = Degradation rate constant (d^{-1}).
[¶]= Degradation was less than 50% of the applied amount.
[#]= Measured pesticide amount below the limit of detection.

Table 4.4 Arrhenius equation, coefficients of determination (r^2), and activation energies for five pesticides at three temperatures, SD in parentheses.

Pesticide	Arrhenius equation†	r^2	Activation energy (KJ mol^{-1})
Tribenuron-methyl	$ln\ k$ = -7.364(1/T) + 22.35	0.972	61.22 ± (0.69)
Thifensulfuron-methyl	$ln\ k$ = -7.721(1/T) + 25.15	0.990	64.19 ± (0.72)
Thiencarbazone-methyl	$ln\ k$ = -3.187(1/T) + 6.81	0.964	26.49 ± (0.30)
Pyrasulfotole	$ln\ k$ = -12.702(1/T) + 39.99	1‡	105.6 ± (0.75)
Bromoxynil	$ln\ k$ = -7.364(1/T) + 25.15	0.971	61.19 ± (0.68)

†k = Degradation rate constant (d^{-1}), T = absolute temperature (degree Kelvin).
‡= Activation energy calculated using two temperatures (13 and 20 ^{o}C).

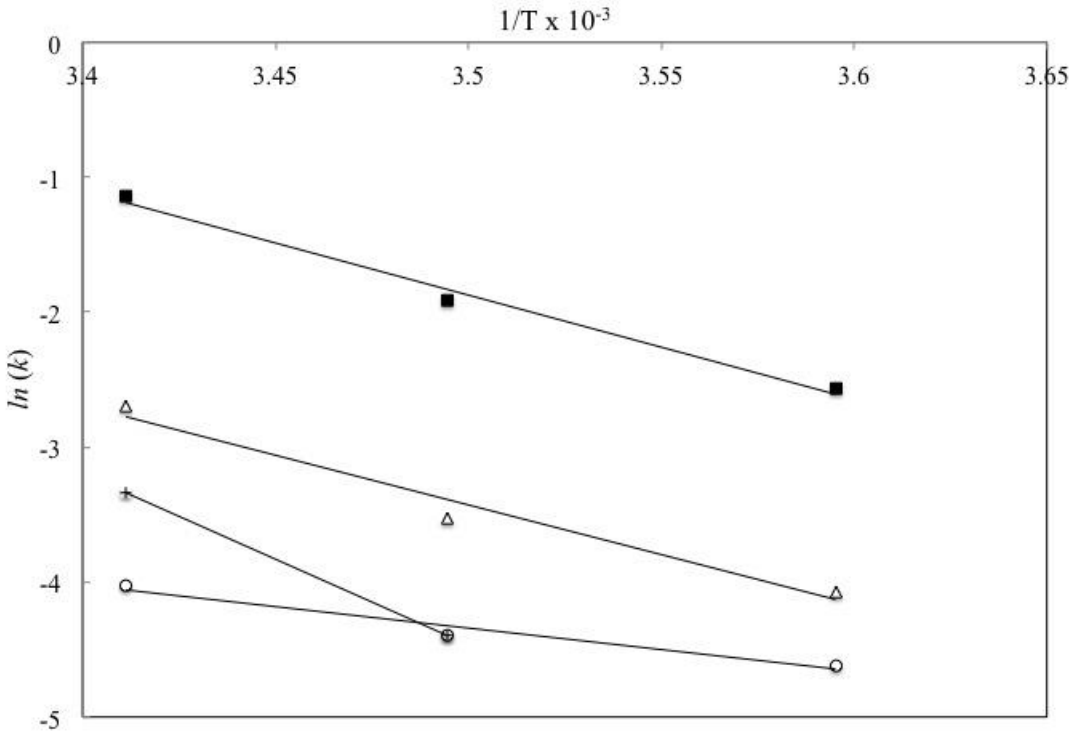

Figure 4.3 Arrhenius diagrams for 5 pesticides in a biobed mix at pH 7.4. The slope indicates activation energies obtained from the natural log of the degradation constant (k) plotted against the reciprocal of the temperature (degree Kelvin). Δ tribenuron-methyl and bromoxynil; ■ thifensulfuron-methyl; o thiencarbazone-methyl; □ pyrasulfotole.

chemical class.

The degradation of sulfonylurea herbicides in soil occurs primarily by chemical hydrolysis and secondarily by microbial action (Fahl et al., 1995). Hydrolytic degradation is favoured in soils with lower pH, whereas in neutral to basic soils microbial degradation predominates. Both processes combined give typical field degradation rates of 1 to 6 weeks depending on soil type, location and the herbicide in question (Cranmer et al., 1999; Menne and Berger, 2001). The general structure of the sulfonylurea herbicides (R'-SO_2NH-CONH-R) is made up of two R groups attached to either side of the sulfonylurea linkage. The R group attached to the sulfur atom of the sulfonylurea can either be an aliphatic, aromatic, or heterocyclic group, whereas that attached to the N atom can be a traizine or pyrimide (Cessna et al., 2010). Their degradation in soil is directly related to their chemical structure and mainly the ionization of the sulfonylurea bridge (Pons and Barriuso, 1998).

Among the sulfonylurea herbicides studied, thifensulfuron-methyl had the fastest degradation rate at all temperatures. Degradation of thifensulfuron-methyl in soil is attributed to deesterification of the parent molecule to the inactive thifensulfuron acid (Smith et al., 1990; Brown et al., 1997). Microorganisms are primarily responsible for the transformation process at a neutral soil pH (Brown et al., 1997). The first step in the degradation process is the hydrolysis of the ester bond. In acidic conditions, (pH 4) the major hydrolysis pathway for thifensulfuron-methyl is by ring cleavage. The half-life of thifensulfuron-methyl increased from 2.2 to 9.1 d with a decrease in temperature from 20 to 5 ^{o}C, respectively, in this study. Rapid degradation of thifensulfuron-methyl has been associated with increases in temperature between 20 and 35 ^{o}C (Cambon et al., 1998). However, temperature affects not just biological processes, but chemical processes as well. Cambon et al. (1998) reported both biological and chemical processes occurring at 20 ^{o}C in a Salanque soil (clay 19.6%, silt 55.6%, sand 19%, OM 1.5%, and pH 7.8) as opposed to just biological degradation in the Saint-Nazaire soil (clay 22%, silt 49.3%, sand 24.3%, OM 2%, and pH 6.3). The rapid degradation of thifensulfuron-methyl observed in the biobed mix could be the result of both biological and chemical processes. Hence, the high degradation cannot be attributed to microbial degradation only. Therefore, more research is needed to determine if both biological and chemical

processes are involved in the degradation of thifensulfuron-methyl in the biobed mix or just one of the processes and to what degree. One hundred percent degradation was achieved after 21 and 28 d of incubation at 20 and 13 ^{o}C, respectively. The calculated activation energy (64.19 ± 0.72 KJ mol^{-1}) and temperature coefficient (Q_{10} = 3.0) support the degradation data. The temperature coefficient shows that for every 10-degree increase in temperature, the degradation rate increased by 3-fold. Dinelli et al. (1997) reported activation energy of 34.44 ± 3.60 KJ mol^{-1} for thifensulfuron-methyl in aqueous solution (pH 4). This indicates that at low pH, temperature has little effect on the hydrolysis of thifensulfuron-methyl.

The second most degraded sulfonylurea herbicide was tribenuron-methyl. The half-life increased from 10.3 to 40.5 d as temperature decreased from 20 to 5 ^{o}C. At 5 ^{o}C, more than 50% of the applied amount (6 g kg^{-1}) was degraded after 35 d. This rapid degradation of tribenuron-methyl could be the result of microbial degradation, as other studies in soil have shown (Fahl et al., 1995; Wang et al., 2011). The calculated activation energy (61.22 ± 0.69 KJ mol^{-1}) and the temperature coefficient (Q_{10} = 3.2) indicate that its degradation is sensitive to temperature and this is supported by the degradation data (73 and 94% degradation at 13 and 20 ^{o}C, respectively).

Degradation of metsulfuron-methyl in the environment may be caused by both chemical hydrolysis and microbial degradation and the degradation pathways include the cleavage of the sulfonylurea bridge, *O*-demethylation of the methoxy-triazine moiety and triazine ring opening after *O*-demethylation (Sarmah and Sabadie, 2002). Various factors affect the degradation of metsulfuron-methyl in the environment. However, the most important factor that affects both its sorption behaviour and chemical degradation in soil is pH, which influences the hydrolysis of metsulfuron-methyl to metsulfuron (Wang et al., 2010). The biobed mix was not capable of degrading metsulfuron-methyl to a greater extent compared to the other pesticides at all temperatures. During the 35-d study period, 29, 33, and 38% of the applied amount (6 g kg^{-1}) was degraded at 5, 13, and 20 ^{o}C, respectively. This was not surprising because at neutral pH metsulfuron-methyl has been found to persist in soil than at acidic pH (Wang et al., 2008). Degradation of metsulfuron-methyl under laboratory conditions (20 ^{o}C) using Prairie soils produced half-life values of 70, 102 and 178 d for clay loam (pH 5.2), sandy loam (pH 6.8), and clay (pH 7.5)

respectively (Smith, 1986). The half-life of metsulfuron-methyl could not be calculated in this study because degradation was too slow at all temperatures. However, by extrapolation, the estimated half-life values were 110.0, 108.3, and 96.3 d at 5, 13, and 20 ^{o}C, respectively. Results found in this study for metsulfuron-methyl showed that its degradation could be pH-dependent as studies in soil have indicated (James et al., 1995; Walker and Jurado-Exposito, 1998; Berglöf et al., 2003). The biobed mix pH (7.4) will favour a slow degradation of metsulfuron-methyl. More degradation may have occurred if the incubation period was extended. Results for metsulfuron-methyl show that maybe the biobed mix used in this study is not suitable for its degradation. Therefore, more research is required to confirm the degradation or non-degradation of metsulfuron-methyl in different biobed mixes.

The degradation of thiencarbazone-methyl at all temperatures plateaued after 14 d of incubation with very little additional degradation occurring to the end of the study period (Figure 4.2 d). Thiencarbazone-methyl is degraded in soil by microorganisms to form three major metabolites: thiencarbazone-carboxylic, thiencarbazone-sulfonamide and thiencarbazone-MMT, which are persistent under aerobic conditions (Health Canada, 2010). The calculated half-life values were 70.1, 55.9, 38.7 d at 5, 13, and 20 ^{o}C, respectively. The half-life values are within the range of those reported in soil. Thiencarbazone-methyl had the lowest activation energy (26.46 ± 0.3 KJ mol^{-1}) of all the studied pesticides (Table 4.10). This suggests that the degradation of thiencarbazone-methyl was not sensitive to temperature. This is evident with the percentage of the applied amount of thiencarbazone-methyl that degraded at all temperatures i.e., 65.6 and 76.8% at 5 and 20 ^{o}C, respectively. Furthermore, the temperature coefficient (Q_{10} = 1.8) supports the degradation data. A 10-degree increase in temperature will only increase the reaction rate by 1.8 fold. Hence, degradation of thiencarbazone-methyl could be the result of other factors such as moisture, pH, and chemical hydrolysis as thiencarbazone-methyl is structurally similar to sulfonylurea herbicides (Santel, 2012).

Pyrasulfotole is degraded in soil through the cleavage of the phenyl ring to form benzoic acid and pyrazole heterocycle metabolites. It is degraded solely by microbial activity because no degradation was reported in sterile soil. Half-life values ranged from 11 to 72 d at 25 ^{o}C at 75% WHC in a variety of soils (Kaune et al., 2008). In this study,

half-life values for pyrasulfotole were 19.5 and 56.4 d at 20 and 13 ^{o}C, respectively. These half-life values obtained in this study lies within the range of those reported by Kaune et al. (2008). By the end of the 35-d incubation period, 41, 52, and 77% of the applied amount (6 g kg^{-1}) was degraded at 5, 13, and 20 ^{o}C, respectively. The high activation energy (105.6 KJ mol^{-1}) indicates that the degradation of pyrasulfotole is very sensitive to temperature compared to the rest of the pesticides. Furthermore, the calculated temperature coefficient (Q_{10} = 4.8) indicates that for every 10-degree increase in temperature, the reaction increased by 4.8 fold. However, its degradation in the biobed mix did not reflect the high activation energy and temperature coefficient, indicating that other factors such as pH, OM, soil type, moisture, and diversity of the microbial population could play a role in its degradation. In a loam sand soil (pH 5.6, %OC 1.2, temperature 25 ^{o}C, and 75% WHC), silt loam soil (pH 7, %OC 4.7, temperature 25 ^{o}C, and 75% WHC), and sand loam soil (pH 6.1, %OC 1.4, temperature 20 ^{o}C, and 50% WHC) the half-life values for pyrasulfotole were 11, 72, and 23 d, respectively (Kaune et al., 2008). The biobed mix used in this study (pH 7.4, % OC 4.8, 70% WHC) gave a half-life value of 19.5 d at 20 ^{o}C.

Degradation of bromoxynil in soil is mainly by microorganisms to form 3,5 dibromo-4-hydroxybenzamide and 3,5-dibromo-4-hydroxybenzoic acid (Golovleva et al., 1988). The main detoxification mechanism in soil is through the microbial hydrolysis of the nitrile group to form the amide and subsequently to carboxylic acid. Degradation of bromoxynil in soil is rapid and is influenced by temperature among other factors. Smith (1971) reported rapid degradation of bromoxynil at 25 ^{o}C compared to 18 ^{o}C similarly in the current study. The half-life of bromoxynil was reduced from 40.5 to 10.3 d as the temperature increased from 5 to 20 ^{o}C. The calculated activated energy (61.19 ± 0.68 KJ mol^{-1}) and temperature coefficient (Q_{10} = 3.3) further support the degradation data. It should be noted that the amount of bromoxynil used in this study was 33.6 mg kg^{-1} as opposed to 6 mg kg^{-1} for the rest of the pesticides. This high concentration was used because pyrasulfotole and bromoxynil are formulated together and pyrasulfotole has a low application rate (31.13 g ha^{-1}). This indicates that the biobed mix is able of degrading high concentration of bromoxynil.

4.5. Conclusion

The degradation of all pesticides in this study increased with an increase in temperature and incubation time. More than 99% of applied amount of 2,4-D, bromoxynil, and thifensulfuron-methyl at both 13 and 20 ^{o}C and 96% at 5 ^{o}C for 2,4-D, and thifensulfuron-methyl, respectively, was degraded in the biobed mix. With the exception of metsulfuron-methyl, with a degradation rate of 38% at 20 ^{o}C, the biobed mix was able to degrade more than 76% of applied a.i. of thiencarbazone-methyl and pyrasulfotole at 20 ^{o}C. The Arrhenius equation and temperature coefficient showed that the degradation of five pesticides of the seven except metsulfuron-methyl and pyrasulfotole were temperature dependent.

Results obtained in this study show that the biobed mix is capable of degrading a mixture of pesticides with contrasting physiochemical properties applied to it as a mixture with the exception of metsulfuron-methyl, which is generally degraded through chemical hydrolysis at low pH. More research is therefore needed in the case of metsulfuron-methyl degradation in the biobed mix.

These results show that high degradation of pesticide in the biobed mix (due to the robust microbial population) should be expected during warm conditions (summer) compared to spring. Hence the timing of pesticide application to the biobed mix is important if maximum degradation is to be achieved under Saskatchewan climatic conditions.

5.0 COMPARISON OF TWO DESIGNS OF FIELD BIOBEDS ON PESTICIDE DEGRADATION

5.1 Introduction

Frequent monitoring of environmental waters around the world has shown that contamination with pesticide residues is common (Wauchope, 1978; Waite et al., 1992; Kolpin et al., 1998; Donald et al., 2007). Such contaminated water could end-up as irrigation and drinking waters with potentially harmful effects on the environment, crops, and human health (Kreuger and Törnqvist, 1998). If such water is intended for human consumption, then the need for treatment is inevitable and expensive. In some rural areas, treatment facilities may not be present and that could pose a potential health hazard. In the prairie provinces of Canada, there are more than 100,000 dugouts (ponds) used by the local inhabitants as their main source of drinking water (Cessna and Elliot, 2004).

Loading, mixing and washing sites are considered a primary target for reducing the amount of pesticide leaving the farmyard to surface water and groundwater (Fogg et al., 2004b). Loading sites are often covered with sand and/or gravel with little ability to adsorb and degrade spilled pesticides. This increases the risk of pesticide leaching to surface water and groundwater or directly into wells or well borings (Henriksen et al., 2003). Studies conducted in Denmark (Helweg et al., 2002; Debear and Jaeken, 2006), the UK, Belgium, and Sweden (Spliid et al., 2006) all identified direct losses from loading sites as the main source of surface water and groundwater contamination by pesticides accounting for 40 to 90% of such contamination (De Wilde et al., 2010). A study conducted in Denmark sampled soil 6 to 10 m below a site previously used as a loading and washing site, and showed mecoprop and dichlorprop at concentrations of 139 and 677 µg L^{-1}, respectively (Henriksen et al., 2003). At a depth of 2 to 4 m dichlorprop and 2,4-D were at concentrations of 750 and 800 µg L^{-1}, respectively, well above the established limit of 0.1 µg L^{-1}. In a survey of a former loading site (decommissioned for at least 15 yr), some pesticides have followed the groundwater for at least 60 m (Henriksen et al., 2003).

Even with the best agricultural practices and trained personnel, spills are inevitable (Fogg et al., 2004c). A small spill of concentrated formulation could contain 1 g a.i. and this would require 10,000,000 L (10,000 m^3) of water to dilute it to the

acceptable limit of 0.1 µg L^{-1} for any one pesticide in drinking water (Vischetti et al., 2004). Therefore, the need to supplement best management practices with on-site bioremediation systems such as the biobeds cannot be ignored. A biobed is a simple on-farm construction intended to collect and degrade spills arising from operational activities around loading and washing areas (Torstensson and Castillo, 1997). Over the years, there have been some modifications to the original below-ground biobed (BGB) design to adapt it to local climatic conditions in countries were biobeds have been studied. For example, the inclusion of an impermeable membrane underneath the biobed and the use of activated carbon filter to remove any pesticides present in the leachate draining from the biobed (Fogg et al., 2004c). One such modification is the new above-ground biobed (AGB) currently being tested in Saskatoon, SK.

The sulfonylurea herbicides, (metsulfuron-methyl, tribenuron-methyl, and thifensulfuron-methly) used in this study are characterized by broad-spectrum weed control at very low use rate. They are degraded in soil through a combination of hydrolysis of the sulfonylurea-bridge and microbial degradation. Hydrolysis is significantly faster under acidic (pH 5) than alkaline (pH 8) conditions (Brown, 1990). Under field conditions, a half-life of 1 to 6 weeks has been reported depending on soil type, location, and the herbicide used (Brown, 1990). Metsulfuron-methyl is known to persist in soil, with about 48% of an applied amount forming bound residues in soil (Ye et al., 2003). Its persistence in soil depends on OM, pH, and environmental factors (Wang et al., 2008). Tribenuron-methyl is degraded in soil through hydrolysis of the sulfonylurea bridge, which is pH dependent. Half-life values of 1 to 6 d have been reported in non-sterile soil (pH 4.3, 1% OM) (Bhattacharjeel and Dureja, 2002). Thifensulfuron-methyl is very susceptible to microbial degradation in soil with a reported half-life ranging from 0.75 to 3.5 days under various soil types, temperature and moisture conditions (Brown et al., 1997). Metsulfuron-methyl, tribenuron-methyl and thifensulfuron-methyl were included in this study due to their average annual usage in the Canadian prairies, their detection in surface water and groundwater (Cessna et al., 2006; Donald et al., 2007), mode of degradation (Cranmer et al., 1999), and their solubility in water, and high mobility in soils (Fahl et al., 1995).

Thiencarbazone-methyl and pyrasulfotole are two herbicides recently introduced

to Canadian farmers. They are used in the control of grass and broadleaf weeds in spring and autumn production in western Canada. Thiencarbazone-methyl is degraded in soil through microbial degradation and the parent molecule and its metabolites do not bind to soil particles (Health Canada, 2010). As a result, they have the potential to leach into groundwater or to be transported in run-off into surface water. Pyrasulfotole and its main metabolite (pyrasulfotole-benzoic acid) are moderately persistent in soil. It is estimated that 19% of an applied amount is carried over to the next cropping season (Health Canada, 2010). It is degraded in soil through microbial degradation (Kaune et al., 2008). These two herbicides were selected on the basis that no studies have been carried out regarding their mobility and/or persistence in the biobeds.

2,4-dichlorophenoxyacetic acid has been widely used across Canada to control post-emergent weeds in cereal production for over 60 yr. It is degraded in soil primarily through microbial degradation (Wilson et al., 1997) to form 2,4-dichlorophenol (Gonod, et al., 2006). 2,4-D dimethylamine salt was included in this study due to its frequent detection in surface water and groundwater across Canada (Grover et al., 1997).

5.1.2 Objectives

The objectives of this study were: 1) to compare the performance of two designs of field biobeds, the traditional BGB and a newly designed AGB with regards to pesticide leaching and degradation, water balance, and temperature, and 2) to recommend the construction of a biobed suitable for operation under Saskatchewan climatic conditions.

5.2 Materials and Methods

5.2.1 Test chemicals

For a complete description of test chemicals and their physicochemical properties, see section 4.2.2.

5.2.2 Preparation of biobed mix

The biobed mix used in this study was prepared by mixing topsoil, composted cattle manure and chopped wheat straw (1:1:2, v/v/v). For a complete description of the biobed mix preparation and chemical properties of the biobed mix, compost, topsoil and

straw see section 3.2.2.

5.2.3 Description of study site

Field biobeds were established at the AAFC research farm (52°8'55" N and 106°34'31" W) in Saskatoon. The site is located within the Prairie Ecozone of Saskatchewan. It is a flat landscape within the Moist Mixed Grassland region and characterized by glacio-lacustrine deposit. It is a Dark Brown Chernozemic soil dominated by a heavy clay soil texture. January and August are the coldest and hottest months, with daily average temperatures of -16.4 and 18.3 °C, respectively. The 30-yr average precipitation (rainfall and snow) for Saskatoon is 348.5 mm (Environment Canada, 2011).

5.2.4 Construction of field biobeds

5.2.4.1 Construction of the below-ground biobed

The BGB (Figure 5.1) was constructed on August 24, 2009 at the AAFC research farm in Saskatoon. The selected area was elevated enough to prevent any runoff water from entering the BGB during heavy rainfall or snow melt. The area was then mapped and excavated (4.6 x 4.3 x 1 m). The excavation was lined with a 1-mm impermeable membrane (NILEX Civil Engineering Group Ltd., Regina, SK), in order to isolate the system from the surrounding ground thereby preventing any leached pesticide from the biobed from contaminating the environment. A drainage tube (weeping tile) was placed around the bottom of the biobed, inclined at one end and connected to a polyvinyl chloride (PVC-U) pipe (7.7 cm i.d.) that protruded above the ground for leachate collection.

To improve drainage, pea gravel (10 cm) was placed over the weeping tile, followed by a nylon mesh. Filling of the BGB with biobed mix was performed with a front-end loader bucket. An irrigation system was installed on the biobed for easy distribution of pesticides and water.

5.2.4.2 Construction of the above-ground biobed

The AGB (Figure 5.2) was constructed September 18, 2009 at the AAFC research farm in Saskatoon, SK. A similar method as for the construction of the BGB was used for

Figure 5.1 Construction of the below-ground biobed, A) hole lined with impermeable membrane followed by weeping tile, B) addition of 10-cm pea gravel, C) hole filled with biobed mix and D) functional biobed with a layer of brome grass. Photos by Brian Caldwell.

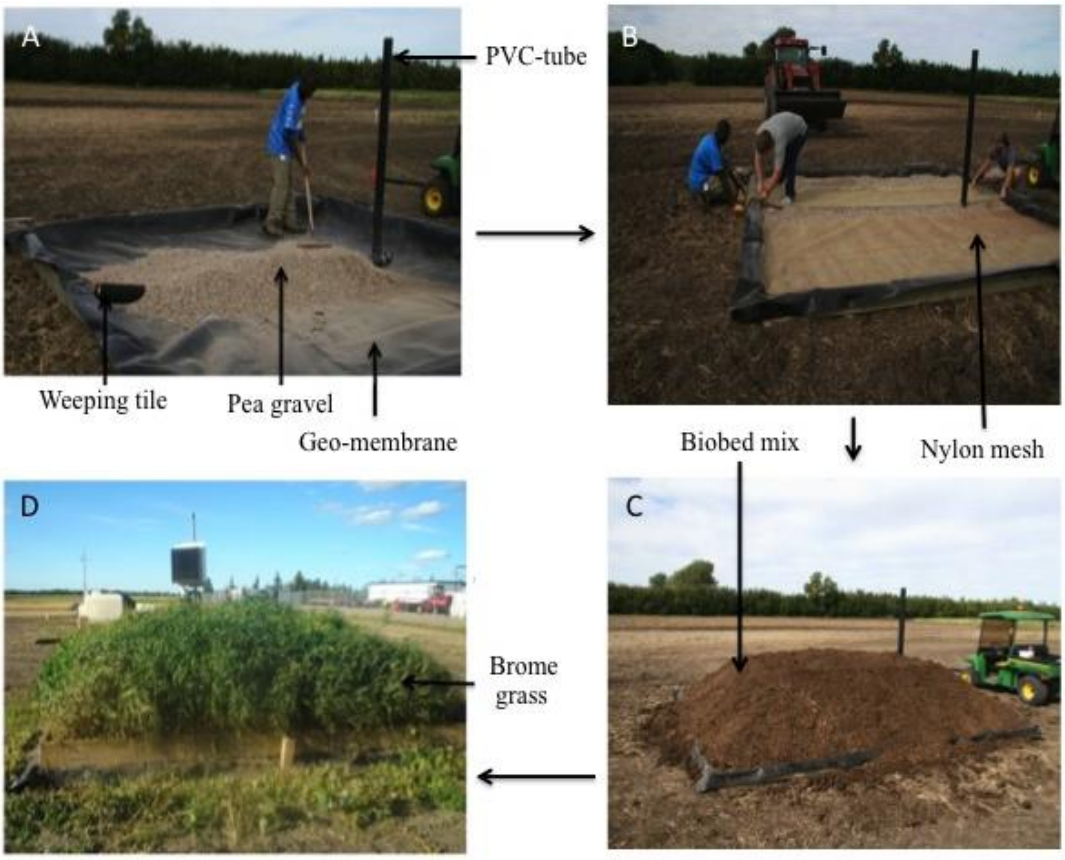

Figure 5.2 Construction of the above-ground biobed, A) elevated area lined with impermeable membrane followed by weeping tile and 10-cm pea gravel, B) pea gravel covered with nylon mesh, C) pea gravel covered with biobed mix and D) functional biobed with a layer of brome grass. Photos by Tom Wolf.

the AGB construction except that no hole was dug. Instead, the area was further raised to prevent surface run-off water from entering the system and the edges supported with wood walls (Figure 5.2). The AGB had as dimensions 4 x 3.8 x 0.75 m. The center of the AGB was approximately 75 cm deep with the edges 40 cm or less. Filling of the AGB was performed with a front-end loader bucket with prepared biobed mix. An irrigation system was also installed on the biobed for easy distribution of pesticides and water.

5.2.4.3 Planting of grass on field biobeds

In preparation for pesticide application, brome grass (*Bromus inermis* spp) was planted on June 15, 2010 on both biobeds. The pre-established brome grass was collected from an adjacent field with a thin layer of topsoil (Spliid et al., 2006). Brome grass was selected due to its deep rooting system.

5.2.4.4 Meteorological station

A mini meteorological station was installed beside the field biobeds in 2009. Precipitation was recorded by means of a precipitation sensor CS 700 (Campbell Scientific, Canada Corp.). Temperature sensors (probe) were buried at 25, 50, 75 and 100 cm in the BGB and 25, 50, and 75 cm in the AGB. The temperature probe 107B (Campbell Scientific Canada Corp.) had a measurement range of -35 to 50 ^{o}C. The moisture content of both biobeds was measured with a time domain reflectometer probe (TDR) in 2010.

5.2.5 Calculation of pesticide concentration

Calculation of pesticide concentration is described in section 4.2.4.

5.2.5.1 Field application of pesticides

The first field application of pesticide was carried out eleven months after the biobeds were established. The six pesticides studied were combined to form a stock solution by dissolving the pesticides in tap water in the order presented in Table 5.1.

For the solid pesticides (metsulfuron-methyl, tribenuron-methyl and thifensulfuron-methyl), each was completely dissolved before the next was added to the mix. Liquid pesticides were measured in a graduated cylinder with the contents emptied into the 10-L jug. The cylinders were rinsed twice with tap water into the 10-L jug. The

Table 5.1 Pesticides, field application rate, a.i. per application, number of applications, and total a.i. applied to field biobeds in 2010 and 2011.

Pesticides	mg a.i. ha^{-1}	mg a.i. per application	No. applications	Total mg a.i.
Metsulfuron-methyl	4446	2500	5	12500
Tribenuron-methyl	7410	2500	5	12500
Thifensulfuron-methyl	3950	2500	5	12500
Thiencarbazone-methyl	5000	2500	5	12500
Pyrasulfotole	31125	2500	5	12500
2,4-D DMA	560000	14000	5	70000
Total mg a.i applied		26500		132500

NB: Bromoxynil was not analyzed for in the field study.

content of the 10-L jug was thoroughly mixed and additional tap water added to the 10-L mark. The contents of the 10-L jug were emptied into a 100-L container half-filled with tap water and twice rinsed with tap water and emptied into the 100-L container. The contents of the 100-L container was thoroughly mixed by shaking. Additional tap water was added to a final volume of 90 L, the contents thoroughly mixed, and the pesticide solution dispensed over the respective biobeds by drip irrigation under gravity. Water was measured with a commercial water metre. Application of pesticide was carried out five times weekly from July 23 to August 19, 2010 and June 22 to July 22, 2011.

5.2.5.2 Sampling of field biobeds

Field biobeds were sampled four times during two growing seasons. In 2010, both biobeds were sampled on July 20 (before pesticide application) and on October 4 (at the end of the first season). In 2011, field biobeds were sampled on June 20 (before pesticide application) and August 19 (end of project). At each sampling time, three spots were randomly selected from each biobed. The grass layer around the selected spots was removed and the biobed sampled at depth of 0 to 15, 15 to 30, 30 to 60, 60 to 90 cm for the BGB and 0 to 15, 15 to 30, 30 to 60 cm for the AGB. Samples were obtained using a Dutch auger (5.5 cm i.d.). The sampled material was immediately placed into labelled plastic bags and stored in a cooler. The bored holes were re-filled with fresh biobed mix. The moisture content of samples was determined by weighing 10 ± 0.03 g, drying for 24 h at 105 ± 2 ^{o}C then re-weighing. The gravimetric moisture content was calculated according to Eq. [3.1]. Following moisture determination, 25 g odw of equivalent sampled material from each depth and biobed was weighed into a 250-mL round bottom flask, corked and placed in a freezer at -20 ± 2 ^{o}C until it was extracted. For microbial biomass determination, duplicate samples (25 g odw equivalent) from each depth were weighed into 100-mL bottles and placed in a freezer at 4 ± 1 ^{o}C for 24 h and then extracted.

5.2.5.3 Leachate sampling

In preparation for pesticide application in 2010, leachate that had accumulated during a period of above-average precipitation was pumped from the BGB from May 21 to July 21, 2010. The volume of the pumped leachate was recorded and the leachate

discarded. For the AGB, leachate was pumped from June 16 to July 21, 2010, because part of the biobed (bottom) was still frozen in May and early June. No samples were collected for pesticide residue analysis from either biobed during this period because no pesticides had been applied. However, on July 23, 2010 (first day of pesticide application), leachate from both biobeds was recorded; duplicate samples were collected for residue analyses and the leachate discarded. Samples for residues analysis were collected from both biobeds during leachate pumping. After the first pesticide application (July 23, 2010), subsequent applications were performed weekly for 4 wk (July 23 to August 19). Prior to each pesticide application, the leachate was sampled (duplicates) for residue analysis and the pumped leachate was stored in near-by tanks. One week after the last pesticide application (August 26, 2010), leachate stored in near-by tanks was re-circulated onto the respective biobeds. For the BGB, leachate that could not be re-circulated due to continued high precipitation was discarded by late October of 2010.

In 2011, there was a slight modification on how the biobeds were treated. To account for pesticide applied in the 2010 season, a carry-over period (May 10 to June 22, 2011 for the BGB and May 31 to June 22, for the AGB) was observed. Some of the biobed material (AGB) was still frozen in early May 2011. During the carry-over period, whenever leachate was pumped from both biobeds, duplicate samples were collected for residue analysis. The pumped leachate was stored in near-by tanks. Leachate collected during the carry-over period was re-circulated onto the respective biobeds prior to pesticide application on June 23, 2011. The BGB was covered during most of the pesticide application period because of heavy rainfall. Furthermore, leachate stored in near-by tanks during the pesticide application period was re-circulated onto the respective biobed between pesticide applications. Leachate samples for residue analysis were kept in a freezer at -20 ± 2 ^{o}C and no further treatment was performed prior to chemical analyses.

5.2.5.4 Microbial biomass-C determination

Microbial biomass-C was determined as described in section 3.2.8.

5.2.5.5 Determination of field biobeds bulk density

The bulk density of field biobeds (depth 0-15 cm) was determined on May 25, 2011. For both biobeds, three spots were randomly selected and the grass cover removed.

Using a hand sledge, a metal ring (height 5 cm and radius 2.3 cm) was driven into the biobed. The ring was up-rooted with a trowel and both surfaces cleared with a knife. The content of the ring was emptied into marked plastic bags. The holes were refilled with fresh biobed mix. Samples were weighed and the moisture determined at 105 ± 2 ^{o}C in an oven for 24 h. The moisture content was calculated as mass of moist biobed mix minus mass of oven dry biobed mix divided by wet weight. Field biobed bulk density was calculated as oven dry mass of biobed mix divided by ring volume. The bulk densities for the BGB and AGB were determined to be 0.42 ± 0.07 and 0.32 ± 0.06 g cm^{-3}, respectively. The BGB had a higher bulk density compared to the AGB because it was more compacted probably due to its design.

5.2.6 Pesticides chemical analysis

Pesticides were analysed as described in section 4.2.6.

5.2.6.1 Calculation of pesticide concentration in the leachate

The mass (mass$_t$) of each pesticide in the leachate at each sampling point (t) was calculated using Eq. [5.1]:

$$Mass_t = C_t * V_t \qquad [5.1]$$

Where C_t is the measured concentration of each pesticide at time t and V_t is the volume of leachate collected at each sampling point t.

The percentage of applied pesticide measured in the leachate was calculated using Eq. [5.2]:

$$\% \text{ Recovered} = \left(Mass_{td} / Total\ applied\ amount\ (theoretical)\right) * 100 \qquad [5.2]$$

Where total mass$_{td}$ is the amount of each pesticide measured in the leachate during the study period and total applied amount (theoretical) is the total amount of each pesticide applied to each biobeds.

In 2011, the amount of pesticide measured in the leachate was adjusted. The

adjustment was made by subtracting the last measured amount of each pesticide during the carry-over period from the amount of each pesticide measured during the last sampling point in 2011 season.

5.2.6.2 Pesticide mass in biobed mix

The mass of each pesticide ($mass_t$) at each depth (0 to 15, 15 to 30, 30 to 60 and 60 to 90 cm) and sampling time (t) was calculated using Eq. [5.3] (Degenhardt et al., 2011).

$$Mass_t = A_B * BD * C_d \quad [5.3]$$

Where A_B is the surface area of the biobed (m^2) for each depth, BD is the bulk density of each biobed, and C_d is the amount of each pesticide measured at each depth and sampling time (t). It was assumed that the bulk density for both biobeds was constant throughout the biobed profile.

Percentage of pesticide retained by each biobed at each depth was calculated using Eq. [5.4].

$$\% \text{ Retained} = \left(Total\ mass_t / total\ applied\ amount\ (theoretical)\right) * 100 \quad [5.4]$$

Where total $mass_t$ is the average amount (n=3) of each pesticide at each depth and total applied amount (theoretical) is the theoretical amount applied to both biobeds.

In 2011, to account for the carry-over amount from the previous year, the percentage of each pesticide amount at each depth was calculated as follows: [(Total amount of pesticide applied in 2011 + measured pesticide amount in the leachate and biobed mix from the carry-over period 2011) – (pesticide amount measured in the leachate and biobed mix in 2011)/(total amount of applied pesticide in 2011 + total amount of pesticides measured during the carry-over period in the biobed mix from 2010 pesticides application)] * 100%.

5.2.7 Statistical and data analysis

Microbial biomass-C data were subjected to a two-way analysis of variance (ANOVA, $P \leq 0.05$) using the CoHort 6.4 software (CoHort 6.4 software, Monterey, CA, 93940, USA), with main factors sampling time (biobed mix) and biobeds (BGB, AGB).

5.3 Results

3.3.1 Results from first year of pesticide application

5.3.1.1 Water balance in field biobeds 2009-2010

The field study was a single experiment carried out over two growing seasons (2010 and 2011). However, because of very different weather conditions in the two growing seasons and differences in when the pesticides were applied, results for each growing season are considered separately. The year 2010 had above-average precipitation (snow and rain) for Saskatoon, the 30-yr average precipitation for Saskatoon is 348 mm and the annual precipitation for 2010 was 635 mm (Environment Canada, 2011). To account for water management in both biobeds, precipitation data from the Saskatoon Research Center (Saskatoon RCS, WMOID: 71496, Climate ID: 4057165) station located at 52’10’25”N and 106’43’08W was used. The meteorological station installed beside field biobeds was disconnected during the winter period. The BGB intercepted 640 mm of precipitation (snow and rainfall) equivalent to 12,649 L, plus an additional 450 L applied with the pesticides, and 615 L through irrigation of grass cover from August 24, 2009 to October 31, 2010.

Prior to pesticide application (July 23, 2010), 5,496 L of leachate was pumped from the BGB (May 21 to July 23, 2010) and discarded. During the pesticide study period (July 23 to October 31, 2010), 2,125 L of leachate was collected of which 1,600 L was re-circulated within the same period of time. By late October of 2010, 525 L of leachate that could not be re-circulated was discarded and water unaccounted for was assumed to have being utilized by evapotranspiration (Figure 5.3).

The AGB received 631 mm of precipitation (snow and rainfall) equivalent to 9,686 L in addition to 450 L applied with the pesticides, and 748 L through irrigation from September 18, 2009 to October 31, 2010. Before pesticide application (July 23, 2010), 1,114 L of leachate was pumped out and discarded. 249 L of leachate collected and re-circulated from July 23 to October 31, 2010, and 9,521 L unaccounted for was

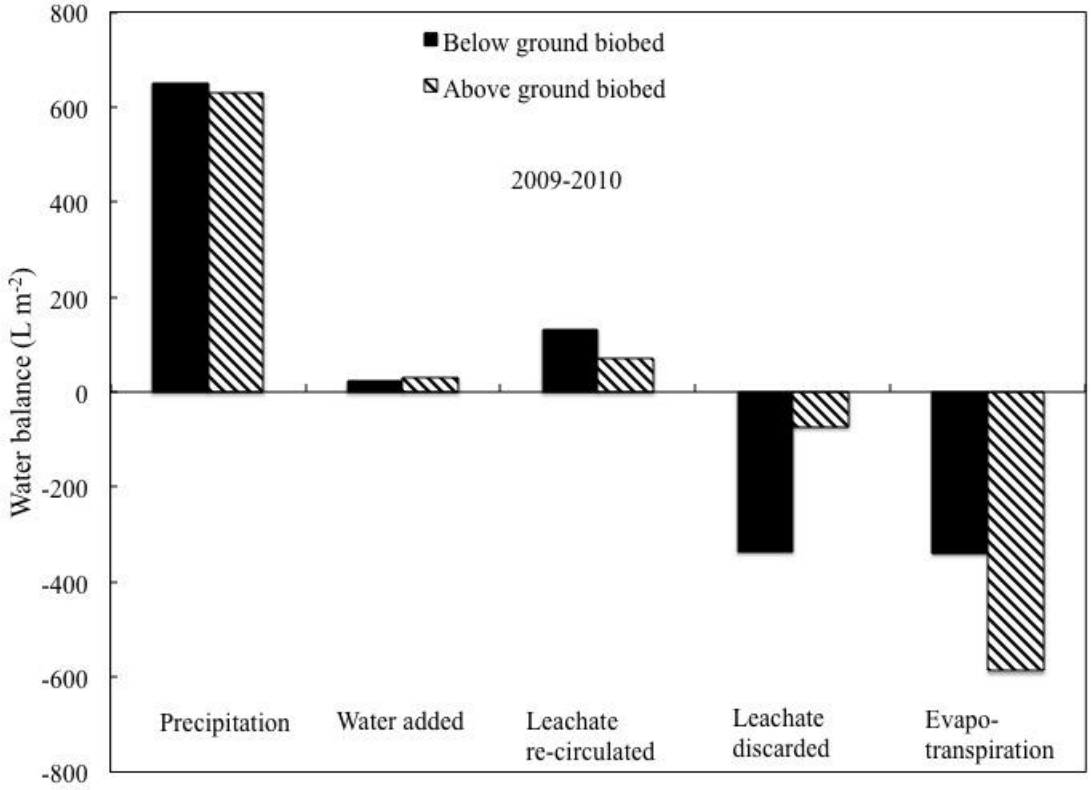

Figure 5.3 Water balance in field biobeds (below- and above-ground biobeds) from September 18, 2009 to October 31, 2010, precipitation (snow and rainfall), water added (addition volume and irrigation), leachate re-circulated and discarded, and evapotranspiration (water unaccounted for).

believed to have been utilized by evapotranspiration (Figure 5.3).

5.3.1.2 Field biobeds moisture content

The moisture content of both biobeds was measured in 2010 with a TDR probe. However, in 2011, moisture measurement was discontinued due to inaccuracy of the TDR probe, probably due to the high OM in the biobed mix that could affect the TDR reading. Therefore, data for biobed moisture are not reported in this study.

5.3.1.3 Field biobed temperature 2009-2010

The temperature probes broke down periodically throughout the study. In 2010, the BGB data were collected from 1 to 9 and 16 to 31 March, 1 to 12 April, 1 to 20 May, and 1 to 24 August with a complete break down occurring in September. For the AGB, data were collected from 26 to 30 June, and 1 to 19 October with the other months having complete data (Figure 5.4). Although the BGB thawed faster than the AGB, looking at the incomplete data plotted, it appears that the AGB reached peak temperature sooner than the BGB (Figure 5.4). The difference between the BGB and AGB in terms of thawing and reaching peak temperatures could be attributed to their respective designs. The AGB temperature at the 50-cm depth was higher than that measured from the 25-cm depth in the BGB in 2010.

5.3.1.4 Leaching of pesticides from field biobeds July 23 to October 4, 2010

In 2010, leachate was collected 10 times over a period of 73 d after pesticide application (July 23 to October 04, 2010). In the BGB, all pesticides under investigation (Table 5.2) were recovered in the leachate 7 d after the first application until the end of the study period, except 2,4-D that was detected only once (28 d) after the first pesticide application.

The cumulative amount (% of applied amount) that leached beneath the BGB from July 29 to October 4, 2010 was 4.2, 0.7, 0.5, 0.4, 0.7, and < 0.01% for metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D DMA, respectively.

In the AGB, all pesticides under investigation were recovered in the leachate 7 d after the first application until the end of the study period (Table 5.3). The most leached

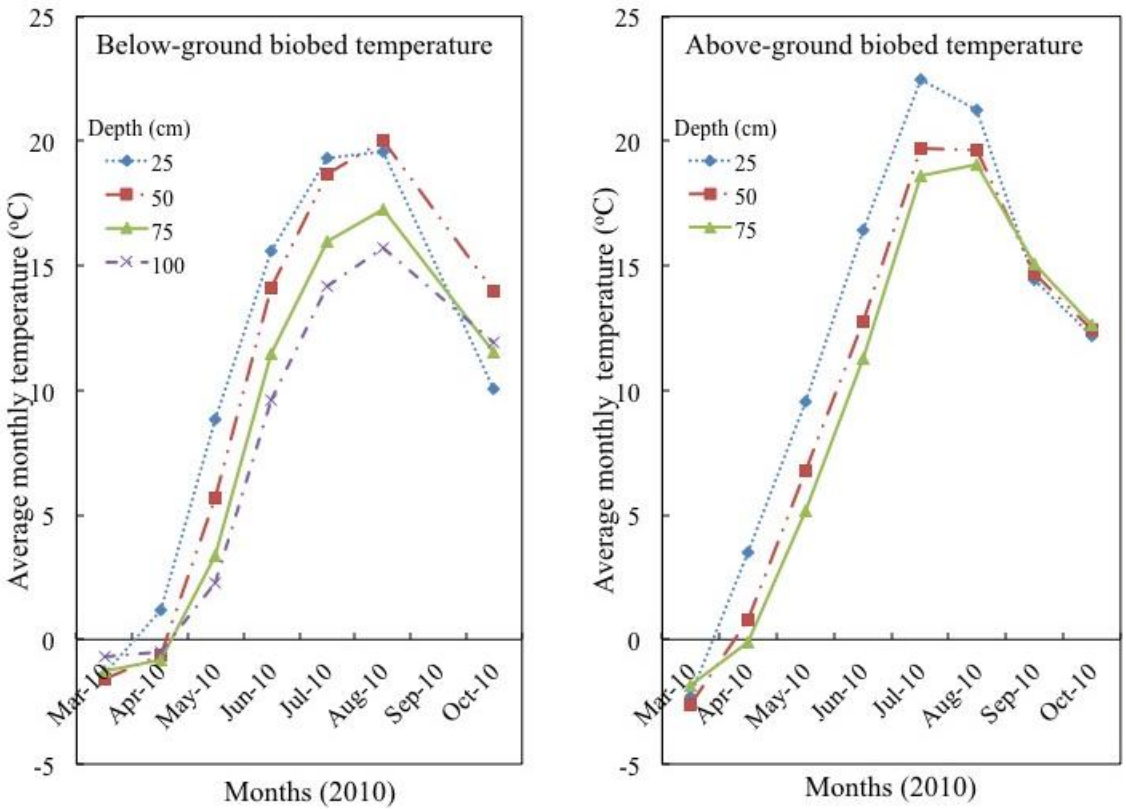

Figure 5.4 Average monthly temperature at various depths (25, 50, 75 and 100 cm). March, April, August and September for the below-ground biobed and June, July and October for the above-ground biobed had incomplete data.

Table 5.2 2010 sampling dates, leachate pumped out (L) and pesticide amount (mg) per sampling time from the below-ground biobed.

		----------Pesticides----------					
Sampling date	Leachate volume (L)	Metsulfuron -methyl	Tribenuron -methyl	Thifensulfuron -methyl	Thiencarbazone -methyl	Pyrasulfotole	2,4-D
		----------Pesticide amount (mg) in leachate----------					
23 July†	15	-‡	-	-	-	-	-
29 July†	53	10.2	1.7	13.6	1.0	3.6	-
4 August†	36	5.1	1.6	6.2	1.1	2.0	-
11 August†	44	4.4	2.2	4.3	1.8	2.2	-
19 August†	106	98.6	7.0	28.1	7.2	7.4	2.5
27 August	76	25.1	3.5	6.4	5.2	4.5	-
1 Sept.	42	12.9	2.3	1.8	2.8	1.8	-
14 Sept.	818	68.1	41.5	-	4.3	32.0	-
27 Sept.	214	156.9	11.6	5.5	16.8	17.9	-
4 October	196	146.8	11.4	3.5	11.1	18.9	-

†Application of pesticides.
‡Measured pesticide amount below limit of detection.

Table 5.3 2010 sampling dates, leachate pumped out (L) and pesticide amount (mg) per sampling time from the above-ground biobed.

		----------Pesticides----------					
Sampling date	Leachate Volume (L)	Metsulfuron -methyl	Tribenuron -methyl	Thifensulfuron -methyl	Thiencarbazone -methyl	Pyrasulfotole	2,4-D
		----------Pesticide amount (mg) in leachate----------					
23 July†	2.0	-‡	-	-	-	-	-
29 July†	3.0	11.0	7.2	6.3	0.7	4.2	8.4
4 August†	3.0	48.0	25.7	24.7	4.7	22.0	-
11 August†	1.5	43.7	19.6	25.0	5.3	19.2	0.5
19 August†	12.5	413.8	169.0	262.3	51.2	150.4	108.6
27 August	38.0	1473.0	581.1	381.1	397.1	664.9	12.6
1 Sept.	15.0	300.7	146.3	80.9	167.0	212.9	0.8
14 Sept.	157.0	968.2	282.4	136.9	250.4	303.2	-
27 Sept.	10.0	69.9	14.5	5.4	13.9	17.8	-
4 October	12.0	75.7	13.1	4.8	25.2	24.4	-

†Application of pesticides.
‡Measured pesticide amount below limit of detection.

pesticide was metsulfuron-methyl while the least leached was 2,4-D DMA (Table 5.3). The cumulative amount (% of applied amount) that leached beneath the biobed from July 29 to October 4, 2010 was 27.2, 10.1, 7.4, 7.3, 11.4, and 0.2% for metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D, respectively. The AGB was more vulnerable to pesticide leaching compared to the BGB.

5.3.1.5 Pesticide degradation in field biobeds July 23 to October 4, 2010

All pesticides under investigation were detected in the BGB matrix at all depths 46 d after the last pesticide application on August 18, 2010. 40.3, 26.0, 2.2, 19.2, 31.9 and 0.6% of metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D DMA, respectively, was recovered in the biobed mix. 58.7, 73.9, 97.8, 80.7, 68.0, and 99.4% of the applied amount of metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D, respectively, was degraded. Metsulfuron-methyl and pyrasulfotole were mainly retained at the 30 to 60 and 60 to 90-cm depths, while for tribenuron-methyl, thifensulfuron-methyl and thiencarbazone-methyl were almost evenly distributed throughout the biobed (Figure 5.5).

In the AGB, 43.8, 21.0, 1.32, 28.2, 20.0, and 0.01% of the applied amount of metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D, respectively, was recovered in the biobed mix 46 d after the last pesticide application (Figure 5.6). 55.6, 78.9, 98.6, 71.6, 79.8 and 99.9% of the applied amount of metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D DMA, respectively, was degraded.

5.3.2 Performance of field biobeds during the carry-over period May to June 2011

5.3.2.1 Pesticide carried over from 2010 season to 2011 in the leachate

Leachate collected from the BGB on May 10, and 11, 2011 contained all six pesticides under investigation (Table 5.4). However, tribenuron-methyl, thiencarbazone-methyl, and 2,4-D were not detected in subsequent leachate samples during the carry-over period that ended on June 22, 2011. Thifensulfuron-methyl concentration decreased

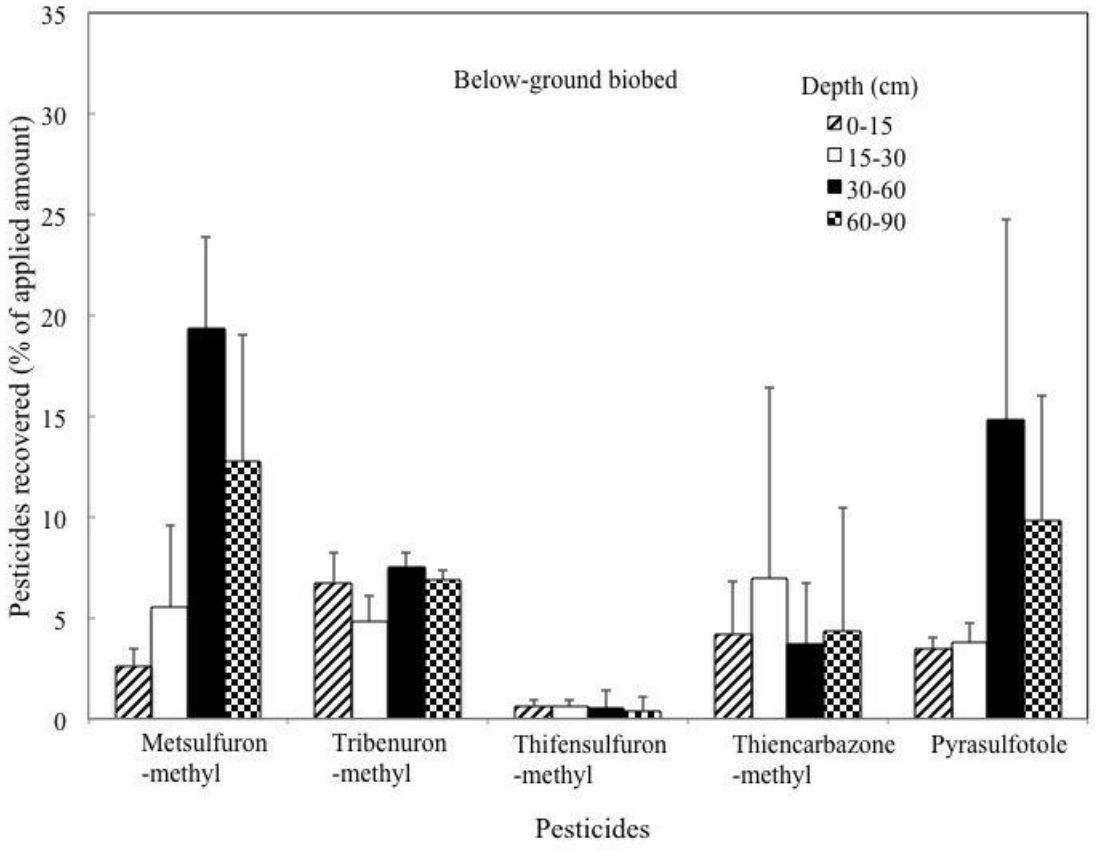

Figure 5.5 Percentage of applied amount of pesticides recovered from the below-ground biobed at various depths (0 to 15, 15 to 30, 30 to 60, and 60 to 90 cm). The biobed was sampled on October 4, 2010, 46 d after the last pesticide application (n=3). Error bars are ± SD.

The amount of 2,4-D recovered from the biobed mix at all depths was less than 0.01% of the applied amount.

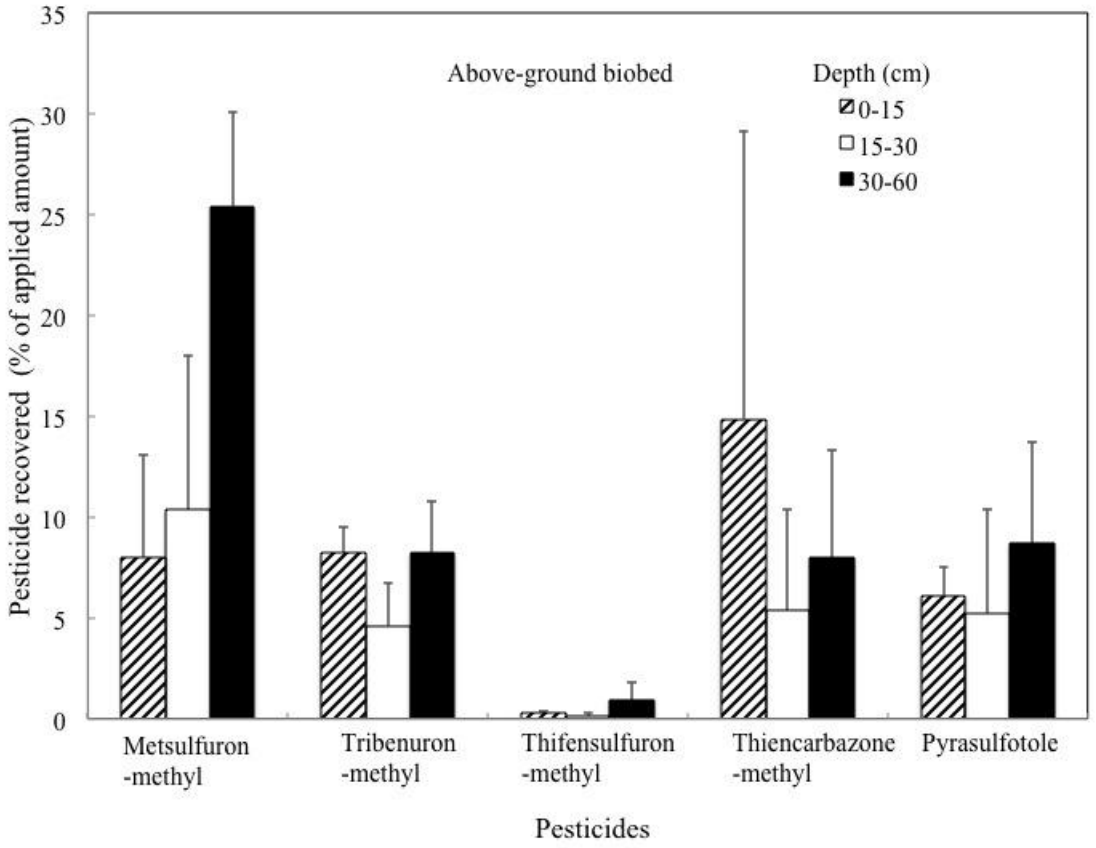

Figure 5.6 Percentage of applied amount of pesticides recovered from the above-ground biobed at various depths (0 to 15, 15 to 30, 30 to 60, and 60 to 90 cm). The biobed was sampled on October 4, 2010, 46 d after the last pesticide application (n=3). Error bars are ± SD.

The amount of 2,4-D recovered from the biobed mix at all depths was less than 0.01% of the applied amount.

Tabl**e 5.4 2011** sampling dates, leachate pumped out (L) and pesticide amount (mg) per sampling time from the below-ground biobed.

		----------Pesticides----------					
Sampling date	Leachate volume (L)	Metsulfuron -methyl	Tribenuron -methyl	Thifensulfuron-methyl	Thiencarbazone -methyl	Pyrasulfotole	2,4-D
		----------Pesticide amount (mg) in leachate----------					
10 May	588	285.2	28.8	51.2	31.2	27.1	16.5
11 May	420	384.3	7.1	31.1	5.0	16.8	4.2
13 May	425	656.2	-†	24.7	-	13.6	-
16 May	190	256.3	-	9.1	-	5.1	-
20 May	125	113.3	-	4.4	-	1.8	-
31 May	140	150.5	-	3.9	-	2.1	-
6 June	68	61.7	-	1.6	-	1.0	-
11 June	135	151.7	-	-	-	2.6	-
15 June	135	165.2	-	-	-	2.3	-
20 June	335	425.8	-	-	-	5.7	-
22 June	106	152.0	-	-	-	1.9	-

†Measured pesticide amount below limit of detection.

with time and by June 6, 2011, it was not detected in the leachate. Metsulfuron-methyl and pyrasulfotole were detected in the leachate throughout the carry-over period, but their respective concentration decreased with time (Table 5.4). From July 23, 2010 to June 22, 2011, the cumulative amount of each pesticide that leached beneath the BGB was 26.6, 1.0, 1.6, 0.7, 1.4 and 0.04% of the applied amount of metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D, respectively.

Some of the biobed mix in the AGB was still partially frozen in early May of 2011 and as a result, first leachate was collected on May 31, 2011 (Table 5.5). The first and second leachates collected on May 31, and June 6, 2011, respectively, contained all six pesticides applied the previous year. Thifensulfuron-methyl was not detected in subsequent samples over the carry-over period (Table 5.5). From July 23, 2010 to June 22, 2011, the cumulative amount of each pesticide that leached beneath the AGB was 34.5, 10.3, 7.5, 8.1, 12.0 and 0.2% of the applied amount of metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D, respectively.

5.3.2.2 Pesticide degradation in the biobeds carry-over period in 2011

Pesticides carried over in both biobed matrices were quantified eight months after the last pesticide application (August 19, 2010). In the BGB, the biobed matrix retained 36, 7, 9, 21, 6, and 0.1% of the applied amount of metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D, respectively. Over the same period of time, 63, 93, 91, 79, 95, and 99% of the applied amount of metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D, respectively, was degraded in the BGB. Tribenuron-methyl, thifensulfuron-methyl and thiencarbazone-methyl, were retained at the 0-15 cm depth, while for metsulfuron-methyl it was at the 30-60 and 60-90 cm depths. The concentration of pyrasulfotole was almost evenly distributed (Figure 5.7).

In the AGB, 14, 0.04, 0.04, 12, 3, and 0.01% of the applied amount of metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D, respectively, was retained by the biobed mix (Figure 5.8). The AGB either retained or degraded 85, 99, 99, 88, 97, and 99% of the applied amount of

Table 5.5 2011 sampling dates, leachate pumped out (L) and pesticide amount (mg) per sampling time from the above-ground biobed.

		----------Pesticides----------					
Sampling date	Leachate volume (L)	Metsulfuron-methyl	Tribenuron-methyl	Thifensulfuron-methyl	Thiencarbazone-methyl	Pyrasulfotole	2,4-D
		----------Pesticide amount (mg) in leachate----------					
31 May	87	117.2	7.4	5.7	17.3	19.0	1.6
6 June	104	168.3	7.1	1.4	22.6	18.0	0.9
11 June	54	112.3	3.1	-†	13.5	7.7	-
15 June	24	56.0	1.5	-	6.1	3.4	-
20 June	225	282.8	3.4	-	30.8	20.0	-
22 June	103	182.5	2.4	-	12.4	9.3	2.2

†Measured pesticide amount below limit of detection.

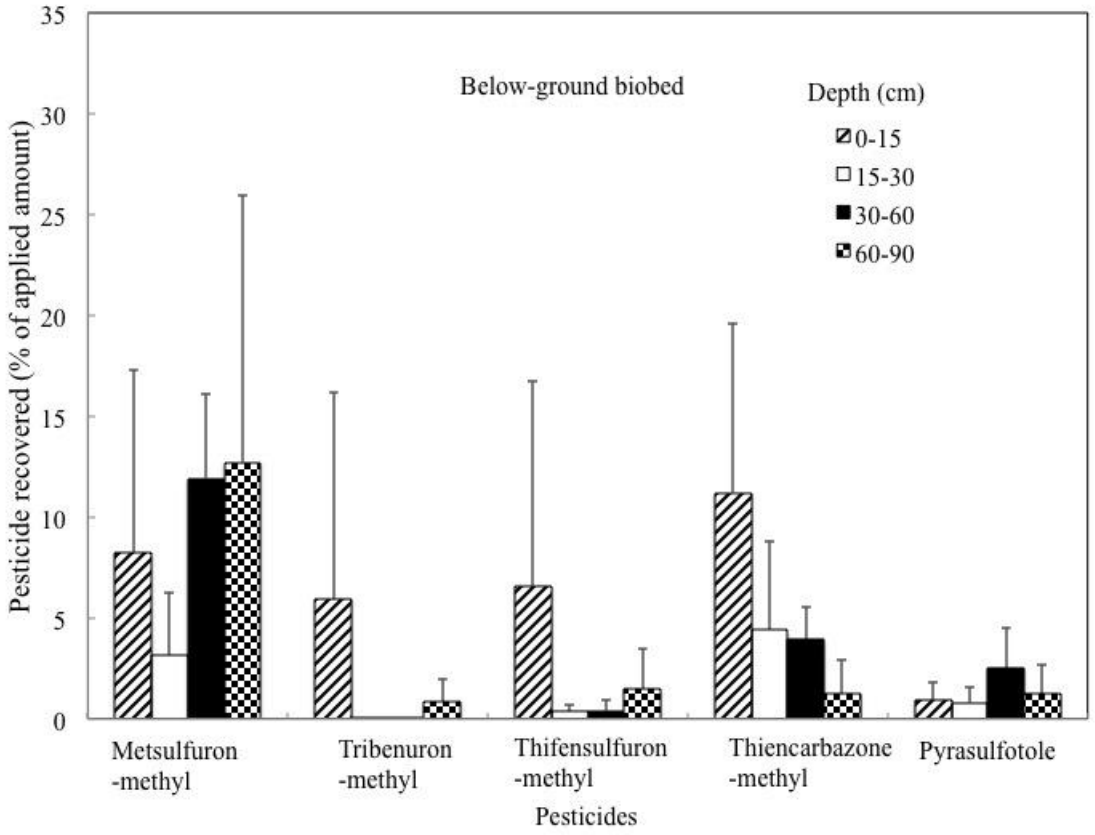

Figure 5.7 Percentage of applied amount of pesticides recovered from the below-ground biobed at various depths (0 to 15, 15 to 30, 30 to 60, and 60 to 90 cm). The biobed was sampled on June 17, 2011, 301 d after first pesticide application (n=3). Error bars are ± SD.

The amount of 2,4-D recovered from the biobed mix at all depths was less than 0.01% of the applied amount.

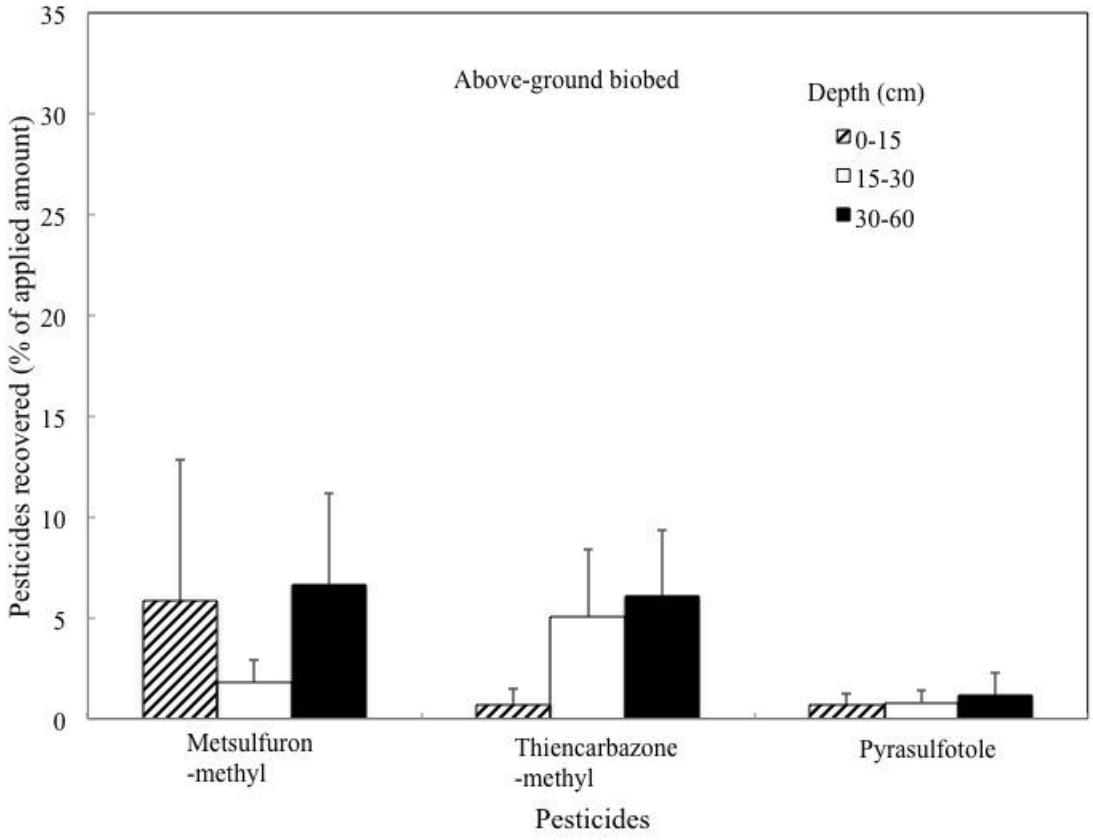

Figure 5.8 Percentage of applied amount of pesticide recovered from the above-ground biobed at various depths (0 to 15, 15 to 30, and 30 to 60 cm). The biobed was sampled on June 17, 2011, 301 d after first pesticide application (n=3). Error bars are ± SD.

The amount of tribenuron-methyl, thifensulfuron-methyl, and 2,4-D recovered from the biobed mix at all depths was less than 0.01% of the applied amount.

metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thicarbazone-methyl, pyrasulfotole, and 2,4-D, respectively. Metsulfuron-methyl was retained mostly at 0-15 and 30-60 cm depths. Thiencarbazone-methyl was retained at the 15-30 and 30-60 cm depths while pyrasulfotole was evenly distributed throughout the biobed profile (Figure 5.8).

5.3.2.3 Pesticide mass balance 2010 pesticide application period

A mass balance for metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D DMA for the BGB (Table 5.6) and ABG (Table 5.7) was performed to determine their environmental fate. The mass balance was calculated using the total amount of pesticide applied (theoretical) to each biobed, the total amount of pesticide measured in the last leachate (as there was re-circulation of collected leachate with time) and the total amount of pesticide extracted from the biobed matrix. For the BGB, with the exception of metsulfuron-methyl (1.2%) less than 0.01% of the other pesticides under investigation leached out (Table 5.6). More than 99% of the applied amount of each pesticide was either degraded or retained by the biobed mix.

For the AGB, with the exception of metsulfuron-methyl (1.5%) less than 0.1% of pesticides under investigation leached out. More than 99% of the applied amount of each pesticide was either degraded or retained by the biobed mix (Table 5.7).

5.3.3 Performance of field biobed in 2011 pesticides application season

5.3.3.1 Water balance in field biobeds 2010-2011

From November 1, 2010 to August 19, 2011, the BGB received 245.4 mm as precipitation (rainfall and snow) equivalent to 4,854 L. Before pesticide application on June 23, 2011, 2,246 L of leachate was pumped out and re-circulated. 450 L of water was applied with the pesticides plus an additional 494 L through irrigation (Figure 5.9). No leachate was discarded from the BGB in the 2010 to 2011 pesticides application season. Leachate marked as discarded (Figure 5.9) refers to the amount of rainfall converted to litre during the period when the BGB was covered.

The AGB received 245.4 mm as precipitation (rainfall and snow) equivalent to 3767 L from November 1, 2010 to August 18, 2011. 494 L of leachate was pumped out

Table 5.6 Mass balances of six pesticides studied in a full-scale below-ground field biobed. Pesticides applied from July 23 to August 19, 2010 (weekly), biobed sampled on June 17, 2011 and last leachate collected on June 22, 2011.

Pesticides	Amount applied (mg)	Amount leached (mg)[†]	Retained by biobed mix (mg)[‡]	% leached	% retained by biobed mix	% degraded[§]
Metsulfuron-methyl	12500	152.0	4500.3	1.2	36.0	62.8
Tribenuron-methyl	12500	-[¶]	853.4	-	6.8	93.2
Thifensulfuron-methyl	12500	-	1095.6	-	8.8	91.2
Thiencarbazone-methyl	12500	-	2389.5	-	20.7	79.3
Pyrasulfotole	12500	1.9	685.0	-	5.5	94.5
2,4-D DMA	70000	-	77.1	-	0.1	99.9

[†]Pesticide amount at last sampling date (Table 5.4 reports pesticides amount in all leachate samples).
[‡]Sum of pesticide amount retained in the below-ground biobed (Figure 5.7 represents pesticides amounts at 0 to 15, 15 to 30, 30 to 60, and 60 to 90-cm depth).
[§]Degradation = 100% - (amount leached + amount retained in biobed mix)%.
[¶]Measured pesticide amount less than 0.05 of the applied amount.

Table 5.7 Mass balances of six pesticides studied a full-scale above-ground field biobed. Pesticides applied from July 23 to August 19, 2010 (weekly), biobed sampled on June 17, 2011 and last leachate collected on June 22, 2011.

Pesticides	Amount applied (mg)	Amount leached (mg)[†]	Retained by biobed mix (mg)[‡]	% leached	% retained by biobed mix	% degraded[§]
Metsulfuron-methyl	12500	182.5	1787.7	1.5	14.3	84.2
Tribenuron-methyl	12500	2.4	-[¶]	-	-	97.6
Thifensulfuron-methyl	12500	-	-	-	-	99.9
Thiencarbazone-methyl	12500	12.4	1480.5	0.1	11.8	88.1
Pyrasulfotole	12500	9.3	332.8	0.1	2.7	97.2
2,4-D DMA	70000	2.2	-	-	-	99.9

[†]Pesticide amount at last sampling date (Table 5.5 reports pesticides amount in all leachate samples).
[‡]Sum of pesticide amount retained in the above-ground biobed (Figure 5.8 represents pesticides amounts at 0 to 15, 15 to 30, and 30 to 60-cm depth).
[§]Degradation = 100% - (amount leached + amount retained in biobed mix)%.
[¶]Measured pesticide amount less than 0.05 of the applied amount.

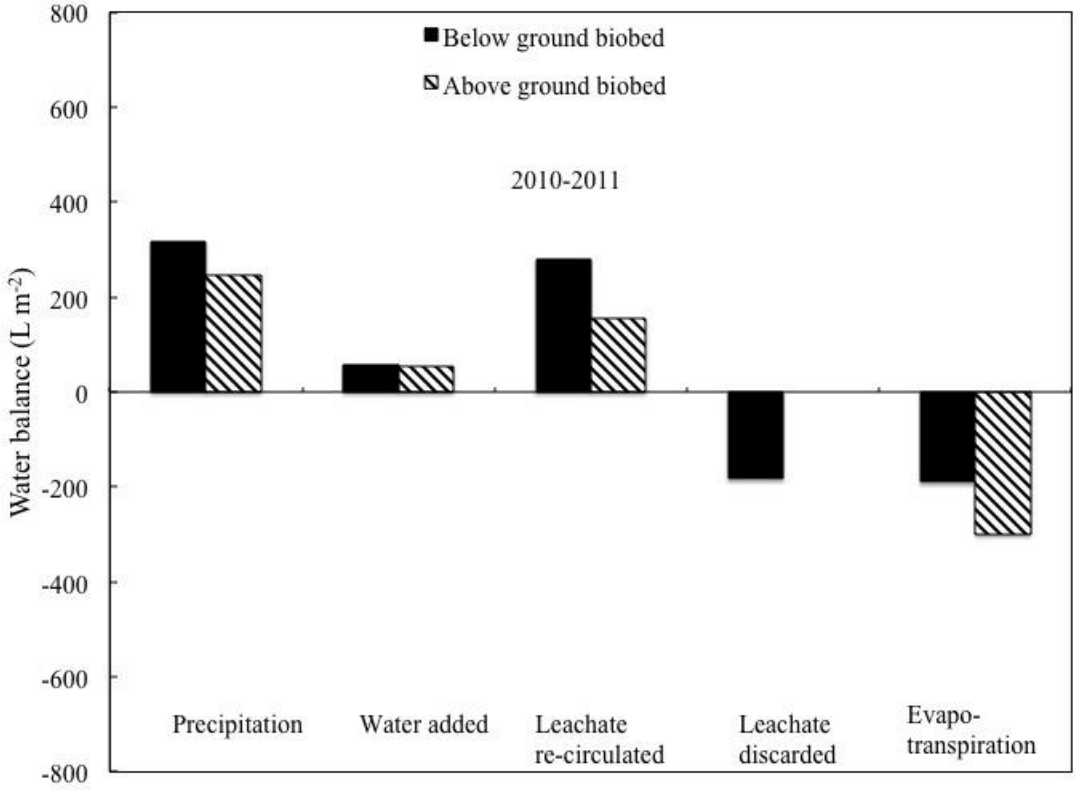

Figure 5.9 Water balance in field biobeds (below- and above-ground biobeds) November 1, 2010 to August 19, 2011, precipitation (rainfall and snow), water added (addition volume and irrigation), leachate re-circulated and discarded, and evapotranspiration (water unaccounted for).

from May 31 to June 22, 2011 and re-circulated. 376 L of water was added to the AGB in the form of irrigation with an additional 450 L applied with pesticides (Figure 5.9).

5.3.3.2 Field biobed temperature in 2011

In 2011, the temperature probe buried in the BGB broke down periodically throughout the months from May to August. The temperature probe buried at 50-cm depth failed throughout 2011 in the BGB. The AGB had incomplete data only for the month of August (1 to 26). However, looking at the incomplete data plotted (Figure 5.10), it appears that the AGB reached peak temperature sooner than the BGB, even though some of the biobed mix at the bottom was partially frozen in early May.

5.3.3.3 Leaching of pesticide in the second year 2011

The reported amounts of pesticide (in the second year) for both biobeds were adjusted by taking into consideration the last measured pesticide amount in the leachate as well as pesticide residue retained by the biobed mix from the previous (2010) pesticide application season.

In 2011, leachate was collected eight times over a period of 57 d from both biobeds. In the BGB, metsulfuron-methyl and pyrasulfotole were detected in the leachate throughout the application period (Table 5.8). Thifensulfuron-methyl was detected in the leachate 3 wk after the first pesticide application until the end of the sampling period. Tribenuron-methyl, thiencarbazone-methyl, and 2,4-D were only detected twice in the leachate during the entire study period. The cumulative loss (% of applied amount) was 14, 0.04, 0.4, 0.1, 0.4, and 0.02% for metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D, respectively.

In the AGB, all pesticides under investigation were detected in the leachate throughout the study period (Table 5.9). The cumulative loss (% of applied amount) was 66, 6, 4.5, 6, 8, and 1% for metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D, respectively.

5.3.3.4 Pesticide residue in the biobed mix second year (2011)

Pesticide residue was estimated 57 d after the first pesticide application. In the BGB, most of the pesticides were concentrated at a depth of 30 to 60 cm (Figure 5.11). The BGB retained 57, 27, 21, 31, 38, and 0.1% of applied amount of metsulfuron-methyl,

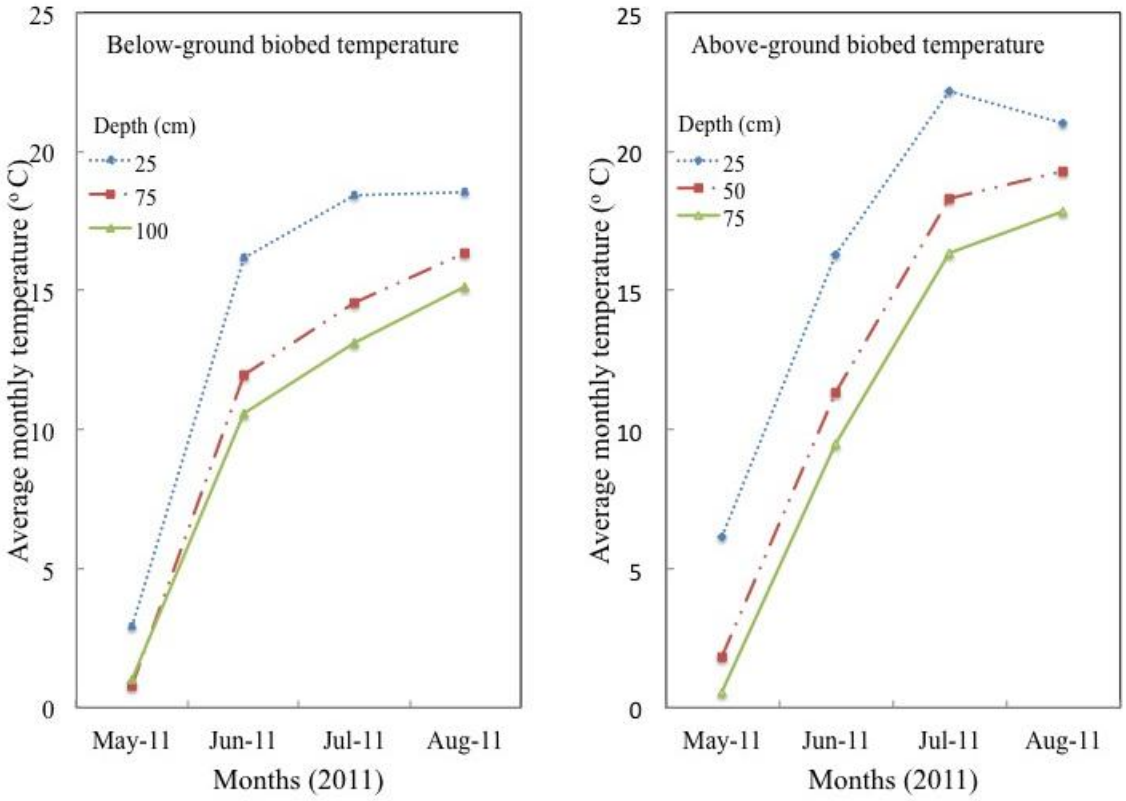

Figure 5.10 Average monthly temperatures at various depths (25, 50, 75 and 100 cm), data from the below-ground biobed are incomplete for all the months while for the above-ground biobed, only the month of August had incomplete data.

Table 5.8 2011 sampling date, leachate pumped out (L) and pesticide amount (mg) per sampling time from the below-ground biobed. First pesticide application occurred on June 23 to July 22, 2011 (weekly) last leachate collected on August 19, 2011.

		----------Pesticides----------					
Sampling date	Leachate volume (L)	Metsulfuron -methyl	Tribenuron -methyl	Thifensulfuron -methyl	Thiencarbazone -methyl	Pyrasulfotole	2,4-D
		----------Pesticide amount (mg) in leachate----------					
27 June†	215	279.5	-‡	-	-	4.7	-
8 July†	53	67.2	-	-	-	0.8	-
15 July†	233	349.3	-	-	-	4.2	-
22 July†	239	405.8	-	13.6	-	8.1	10.3
25 July	112	200.1	-	2.8	-	2.8	-
3 August	258	478.3	1.0	15.0	2.1	15.2	-
12 August	168	324.1	-	4.2	-	4.5	-
19 August	114	267.6	3.8	20.4	4.9	8.1	3.1

†Application of pesticides.
‡Measured pesticide amount below limit of detection.

Table 5.9 2011 sampling date, leachate pumped out (L) and pesticide amount (mg) per sampling time from the above-ground biobed. First pesticide application occurred on June 23 to July 22, 2011 (weekly) last leachate collected on August 19, 2011.

		----------Pesticides----------					
Sampling date	Leachate (L)	Metsulfuron -methyl	Tribenuron -methyl	Thifensulfuron -methyl	Thiencarbazone -methyl	Pyrasulfotole	2,4-D
		----------Pesticide amount (mg) in leachate----------					
27 June†	210	541.0	10.7	4.8	32.6	21.2	12.8
8 July†	108	559.1	81.8	68.0	32.4	53.0	159.1
15 July†	265	1457.5	182.1	136.2	114.0	153.2	127.5
22 July†	343	1917.4	177.3	157.8	151.9	195.9	194.8
25 July	279	1781.7	144.2	127.5	157.4	206.5	79.5
3 August	264	1508.0	63.9	49.9	116.4	154.7	11.4
12 August	147	810.4	26.9	14.3	77.2	90.8	9.3
19 August	184	1006.1	19.1	6.1	109.5	114.3	0.1

†Application of pesticides.

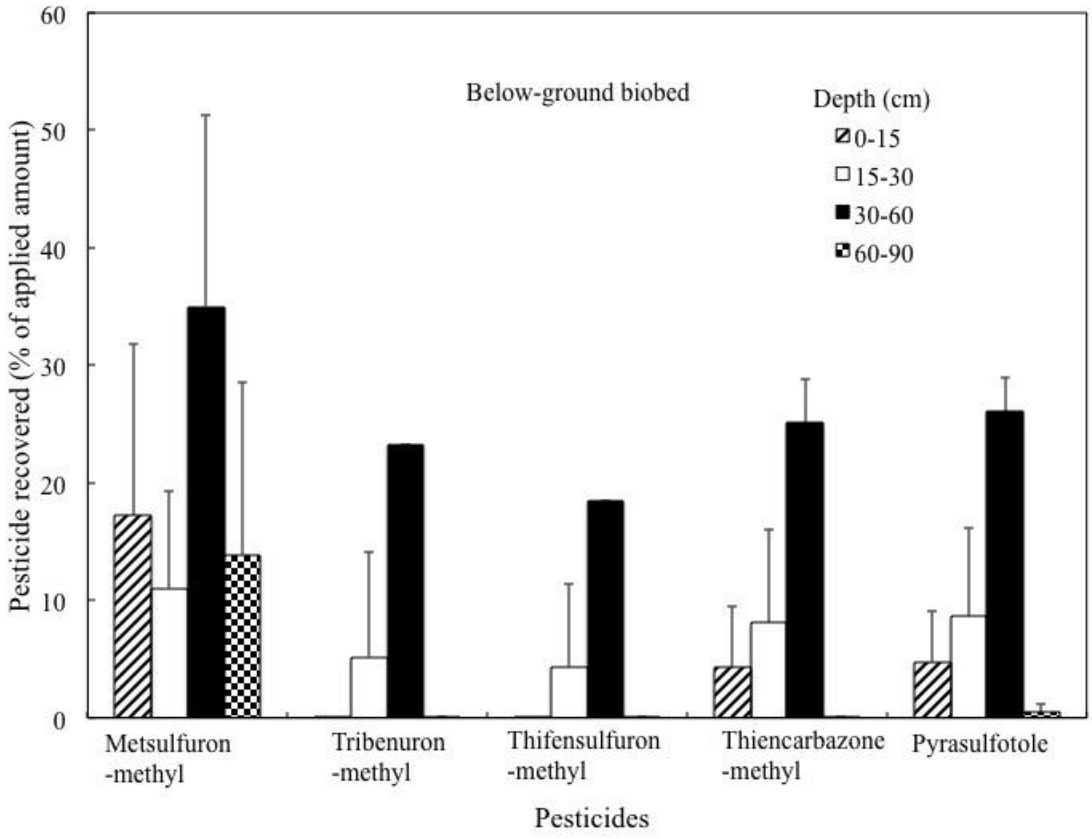

Figure 5.11 Percentage of applied amount of pesticides recovered from the below-ground biobed at various depths (0 to 15, 15 to 30, 30 to 60, and 60 to 90 cm). The biobed was sampled on August 19, 2011, 57 d after first pesticide application (n=3) on June 22, 2011. Error bars are ± SD.

The amount of 2,4-D recovered from the biobed mix at all depths was less than 0.01% of the applied amount.

tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D, respectively.

In the AGB, 28, 0.04, 0.04, 6, 4, and 0.02% of the applied amount of metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D, respectively, was recovered from the biobed mix. Metsulfuron-methyl was concentrated at the depth of 30 to 60 cm, whereas pyrasulfotole and thiencarbazone-methyl were concentrated at the 15 to 30-cm depth (Figure 5.12).

5.3.3.5 Pesticide mass balance second year of study 2011

A mass balance for the second year of pesticide application showed that for the BGB, with the exception of metsulfuron-methyl (2.1%), less than 0.2% of all pesticides under investigation leached out of the system and more than 97% of the applied pesticide was either degraded or retained by the biobed mix (Table 5.10). For the AGB, with the exception of metsulfuron-methyl (7%), less than 1% of pesticides under investigation leached out. More than 93% of applied amount of the pesticide was either degraded or retained by the biobed mix (Table 5.11).

5.3.11 Microbial biomass carbon

Microbial biomass was measured in October after pesticide application in 2010. For the BGB, the estimated MB-C ranged from 0.5 (0 to 15 cm) to 0.3 mg kg^{-1} C (60 to 90 cm) and a significant difference (Table 5.12) was observed between 0 to 15 and 60 to 90-cm depths. In the AGB, there was no significant difference, among the various depths and the estimated MB-C (October) ranged from 0.3 (0 to 15 cm) to 0.2 mg kg^{-1} C (30 to 60 cm).

In 2011, microbial biomass was measured in June and August and the MB-C did not differ significantly at all depths for both biobeds. Generally, there was a significant interaction between the biobed type and MB-C sampling time at the 15 to 30-cm depth (Table 5.12).

5.4 Discussion

5.4.1 Water management and the leaching of pesticides from the below-ground biobed and above-ground biobed

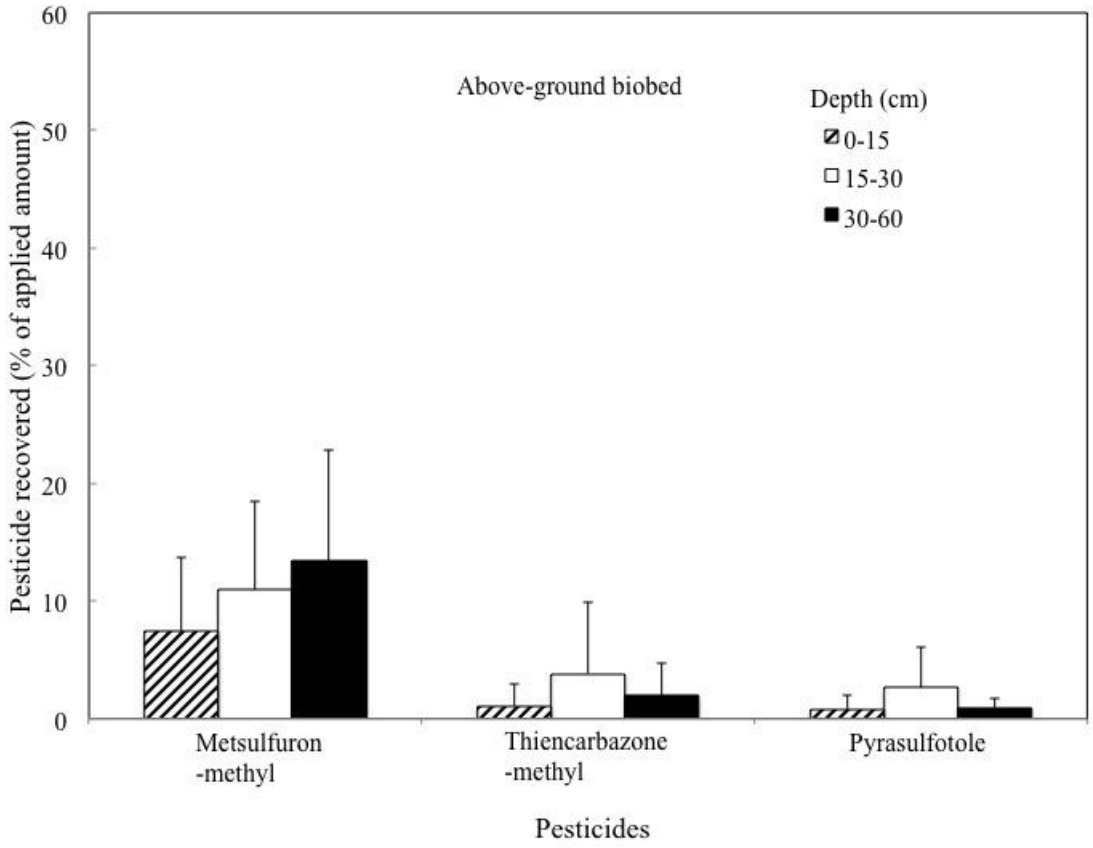

Figure 5.12 Percentage of applied amount of pesticides recovered from the above-ground biobed at various depths (0 to 15, 15 to 30, and 30 to 60 cm). The biobed was sampled on August 19, 2011, 57 d after first pesticide application (n=3) on June 22, 2011. Error bars are ± SD.

The amount of tribenuron-methyl, thifensulfuron-methyl, and 2,4-D recovered from the biobed mix at all depths was less than 0.01% of the applied amount.

Table 5.10 Mass balances of six pesticides studied in a full-scale below-ground field biobed. Pesticides applied from June 23, to July 22, 2011 (weekly), last leachate and biobed sampled on August 19, 2011.

Pesticides	Amount applied (mg)	Amount leached (mg)[†]	Retained by biobed mix (mg)[‡]	% leached[§]	% retained by biobed mix[¶]	% degraded[#]
Metsulfuron-methyl	12500	267.6	9648.6	2.1	56.8	41.1
Tribenuron-methyl	12500	3.8	3561.3	-[††]	26.7	73.3
Thifensulfuron-methyl	12500	20.4	2852.8	-	21.0	79.0
Thiencarbazone-methyl	12500	4.9	4688.0	0.2	31.1	68.7
Pyrasulfotole	12500	8.1	4997.0	0.1	37.9	62.0
2,4-D DMA	70000	3.1	81.3	-	0.1	99.9

[†]Pesticide amount at last sampling date (Table 5.8 reports pesticides amount in all leachate samples).

[‡]Sum of pesticide amount retained in the below-ground biobed (Figure 5.11 represents pesticides amounts at 0 to 15, 15 to 30, 30 to 60, and 60 to 90-cm depth).

[§]Percentage leached equals to amount of pesticide measured in last leachate sampled on June 22, 2011 (Table 5.4) divided by (pesticide applied amount + pesticide amount measured in last leachate sampled on August 19, 2011 (Table 5.8) multiplied by 100%.

[¶]Sum of pesticide amount retained in the below-ground biobed (Figure 5.11 reports pesticides amounts at 0 to 15, 15 to 30, 30 to 60, and 60 to 90-cm depth) divided by (amount applied + pesticide amount carried over in the biobed at all depths (Figure 5.7).

[#]Percentage degraded equals to sum of pesticide amount retained in the below-ground biobed sampled on August 19, 2011 (Figure 5.11 represents pesticides amounts at 0 to 15, 15 to 30, 30 to 60, and 60 to 90-cm depth) divided by (pesticide applied amount + pesticide amount measured in the biobed sampled on June 17, 2011 (Figure 5.11 represents pesticides amounts at 0 to 15, 15 to 30, 30 to 60, and 60 to 90-cm depth) multiplied by 100%.

[††]Measured pesticide amount less than 0.05.

Table 5.11 Mass balances of six pesticides studied in a full-scale above-ground field biobed. Pesticides applied from June 23, to July 22, 2011 (weekly), last leachate and biobed mix was sampled on August 19, 2011.

Pesticides	Amount applied (mg)	Amount leached (mg)[†]	Retained by biobed mix (mg)[‡]	% leached[§]	% retained by biobed mix[¶]	% degraded[#]
Metsulfuron-methyl	12500	1006.1	9648.6	7.0	27.8	65.2
Tribenuron-methyl	12500	19.1	3561.3	0.2	-[††]	99.8
Thifensulfuron-methyl	12500	6.1	2852.8	0.1	-	99.9
Thiencarbazone-methyl	12500	109.5	4688.0	0.8	6.1	93.1
Pyrasulfotole	12500	114.3	4997.0	0.9	4.3	94.8
2,4-D DMA	70000	0.1	81.3	-	-	99.9

[†]Pesticide amount at last sampling date (Table 5.9 reports pesticides amount in all leachate samples).

[‡]Sum of pesticide amount retained in the above-ground biobed (Figure 5.12 represents pesticides amounts at 0 to 15, 15 to 30, and 30 to 60-cm depth).

[§]Percentage leached equals to amount in last leachate sampled on June 22, 2011 (Table 5.5) divided by (pesticide applied amount + pesticide amount measured in last leachate sampled on August 19, 2011 (Table 5.9) multiplied by 100%.

[¶]Sum of pesticide amount retained in the above-ground biobed (Figure 5.12 reports pesticides amounts at 0 to 15, 15 to 30, and 30 to 60-cm depth) divided by (amount applied + amount carried over in the biobed at all depths (Figure 5.8).

[#]Percentage degraded equals to sum of pesticide amount retained in the above-ground biobed sampled on August 19, 2011 (Figure 5.12 represents pesticides amounts at 0 to 15, 15 to 30, and 30 to 60-cm depth) divided by (pesticide applied amount + pesticide amount measured in the biobed sampled on June 17, 2011 (Figure 5.12 represents pesticides amounts at 0 to 15, 15 to 30, and 30 to 60-cm depth) multiplied by 100%.

[††]Measured pesticide amount less than 0.05.

Table 5.12 Effect of time and biobed type on the microbial biomass-C at 0 to 15, 15 to 30, and 30 to 60 cm during 10 months period (October 2010 to August 2011).

	Biobeds					
	Below ground biobed			Above ground biobed		
	Depths (cm)			Depths (cm)		
	0-15	15-30	30-60	0-15	15-30	30-60
Time	----------mg kg^{-1} biobed mix----------			---------mg kg^{-1} biobed mix------		
October 2010	0.5	0.4	0.4	0.3	0.3	0.2
June 2011	0.6	0.6	0.6	0.6	0.6	0.7
August 2011	30.7	29.4	35.0	34.0	34.0	39.0

Source of variation	df	----------Probability (*P*)----------		
		0-5 cm	15-30 cm	30-60 cm
Time (T)	2	< 0.0001	< 0.0001	< 0.0001
Biobed (B)	1	0.6035	0.0097	0.5132
Interaction (T*B)	2	0.7145	0.0026	0.6049

Water management is important for optimum performance of biobeds. Low moisture may limit microbial activity and the amount of pesticide in solution while near saturation conditions can lead to anaerobic conditions that could limit pesticide degradation. A moisture content of about 60% WHC is ideal because it gives enough water for microbial processes, solubilisation of pesticides and free pore space for oxygen to support aerobic processes (Castillo and Torstensson, 2007; Castillo et al., 2008). However, due to poor moisture readings from the TDR probe in 2010, it was discontinued. From August 24, 2009 to October 31, 2010, the BGB received 13,714 L of water. During the same period of time, 6,021 L was collected as leachate and discarded.

The AGB received 10,884 L of water from September 18, 2009 to October 31, 2010. 1,114 L was collected as leachate and discarded. The unaccounted water 7,595 and 9,521 L from the BGB and AGB, respectively, can be attributed to plant usage. The established grass layer on both biobeds did not prevent downward water movement. This suggests that root uptake of water by plants and evapotranspiration are not sufficient to regulate moisture in the biobed, especially if it receives high volumes of water through persistent rainfall, as was the case in 2010 in Saskatoon, SK. Spliid et al. (2006) found similar results where pre-grown established grass was unable to compensate for the downward movement of water in a biobed during the summer period in Denmark. Leaching of pesticides from both field biobeds was affected by the hydraulic loading. In 2010, with more than average precipitation for Saskatoon (635 mm), the cumulative pesticide loss from the BGB was 26.6, 1.0, 1.6, 0.7, 1.4, and 0.04% of the applied amount for metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D, respectively.

In the AGB, the cumulative amount (% of applied amount) of each pesticide that leached beneath the biobed was 34.5, 10.3, 7.5, 8.2, 12.0, and 0.2% for metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D, respectively, in 12 months.

In 2011, the grass cover from either biobeds did not recover very well from the pesticide treatment in 2010. To improve water management and possible pesticide leaching beneath the system, the BGB was covered during the pesticide application period and heavy rainfall. It was difficult to protect the AGB from rainfall due to its

design. The cover placed over the BGB during heavy rainfall and pesticide application period improved the efficiency of the system. With the exception of metsulfuron-methyl (13.8%), less than 0.4% of applied pesticides leached. However, the problem with covering the biobed is that the top layer dries out very quickly and regular irrigation is required. Therefore, covering the biobed may not be a practical solution as the top layer forms a hydrophobic layer that could restrict evaporation and moisture loss (De Wilde et al., 2007). The formed hydrophobic layer could lead to saturation conditions below the 0 to 10-cm layer, and that can inhibit microbial activity responsible for the degradation of the applied pesticides (Castillo et al., 2008). A possible solution is to drain the leachate from the biobed into a near-by tank and later re-introduce it onto the biobed by means of a pump. This could increase the operational costs and may not be attractive to farmers. Moisture could also be managed in the lined BGB by covering it during winter periods to exclude excessive moisture in the form of snowfall.

In contrast, 66.3, 5.7, 4.5, 5.7 7.7, and 0.9% of the applied amount of metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D, respectively, leached beneath the AGB. The vulnerability of the AGB could have been due to its depth (0.25 m shallower then the BGB), absence of a grass layer (second year) and the inability to protect it from rainfall. Results obtained in 2010 and 2011 are in agreement with those reported by Henriksen et al. (2003). The authors reported no mecoprop-P and isoproturon in the leachate when the biobed was covered during the second year after intercepting 450 mm of precipitation compared to 12.9 and 1.4% in the leachate, respectively in the first year when the biobed intercepted 780 mm of precipitation. In a 0.5 m biobed that received 9,747 L m^{-2} (high water loading), 2,797 L m^{-2} (medium water loading), and 486 L m^{-2} (low water loading), 6.3, 0.2, and 0.0% of isoproturon leached out respectively (Fogg et al., 2004b). Results obtained for metsulfuron-methyl in the AGB were better than those reported by Fogg et al. (2004b). In a column experiment with a depth of 0.5 m, 100, 48.3, and 0.2% of metsulfuron-methyl leached out when the columns were subjected to high water loading (1,175 L m^{-2}), medium water loading (688 L m^{-2}) and direct input from rainfall or low water loading (202 L m^{-2}) respectively, compared to 19.3, 18.4, and 0.003% in a column at 1-m depth biobed, and 15.3, 5.9, and 0.0002 in a 1.5-m depth biobed under the same

hydraulic loading. Similar to the current study with metsulfuron-methyl, these studies suggest that the biobed mix is not suitable for metsulfuron-methyl use assuming application every year. These results show that hydraulic loading, and biobed depth are important factors and should be considered in establishing biobeds.

5.4.3 Degradation of pesticides in field biobeds

The biobed mix consists of an active biological matrix that retains pesticides into the OM; microorganisms then degrade the trapped pesticides. In the first year, 62.8, 93.2, 91.2, 79.3, 94.5, and 99.9% of the applied amount of metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D, respectively, was degraded in 12 months (Table 5.6), compared to 84.2, 97.6, 99.7, 88.1, 97.2, and 99.9% in the AGB (Table 5.7). In the second year, 22.2, 73.3, 78.9, 68.9, 62.1, and 99.9% of metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D DMA, respectively degraded in 54 d in the BGB (Table 5.10), compared to 65.2, 99.8, 99.9, 93.1, 94.8, and 99.9% in the AGB (Table 5.11).

During the entire study (two growing seasons), with the exception of metsulfuron-methyl (60.3%), more than 80% of the applied pesticides were degraded in the BGB and less than 0.01% leached except metsulfuron-methyl (1.1%) (Table 5.13). In the AGB, more than 80% of applied pesticides were degraded. With the exception of metsulfuron-methyl (4.0%), less than 0.5% of all pesticides leached (Table 5.14).

The rapid degradation of pesticides in the second growing season could have been as a result of multiple factors such as repeated applications of pesticides, and differences in temperature, application time and microbial biomass. Repeated addition of the same pesticide to the same substrate could result in enhanced degradation due to adaptation and proliferation of specific microorganisms capable of utilizing the pesticide as a source of C and energy (De Wilde et al., 2007).

In this study, pesticides were applied to both biobeds 10 times during two growing seasons and the rapid degradation in the second year could have been as a result of it. The half-life of metalaxyl was reduced from 37 d (first application) to 14 d (second application), to 4 d (third application) in a biobed mix (Vischetti et al., 2008). The half-

Table 5.13 Mass balances of six pesticides studied in a full-scale below-ground field biobed during a two-year period (July 23, 2010 to August 19, 2011).

Pesticides	Amount applied (mg)	Amount leached (mg)[†]	Retained by biobed mix (mg)[‡]	% leached	% retained by biobed mix	% degraded[§]
Metsulfuron-methyl	25000	267.6	9648.6	1.1	38.6	60.3
Tribenuron-methyl	25000	3.8	3561.3	-[¶]	14.3	85.7
Thifensulfuron-methyl	25000	20.4	2852.8	0.1	11.4	88.5
Thiencarbazone-methyl	25000	4.9	4688.0	-	18.8	81.2
Pyrasulfotole	25000	8.1	4997.0	-	20.0	80.0
2,4-D DMA	140000	3.1	81.3	-	0.1	99.9

[†]Pesticide amount at last sampling date (Table 5.8 reports pesticides amount in all leachate samples).
[‡]Sum of pesticide amount retained in the below-ground biobed (Figure 5.11 represents pesticides amounts at 0 to 15, 15 to 30, 30 to 60, 60 to 90-cm depth).
[§]Degradation = 100% - (amount leached + amount retained in biobed mix)%.
[¶]Percentage leached was less than 0.01% of the applied amount.

Table 5.14 Mass balances of six pesticides studied in a full-scale above-ground field biobed during a two-year period (July 23, 2010 to August 19, 2011).

Pesticides	Amount applied (mg)	Amount leached (mg)[†]	Retained by biobed mix (mg)[‡]	% leached	% retained by biobed mix	% degraded[§]
Metsulfuron-methyl	25000	1006.1	3975.1	4.0	15.9	80.1
Tribenuron-methyl	25000	19.1	5.0	0.1	-[¶]	99.9
Thifensulfuron-methyl	25000	6.1	5.0	-	-	99.9
Thiencarbazone-methyl	25000	109.5	854.3	0.4	3.4	96.2
Pyrasulfotole	25000	114.3	545.4	0.5	2.2	97.3
2,4-D DMA	140000	0.1	14.5	-	-	99.9

[†]Pesticide amount at last sampling date (Table 5.9 reports pesticides amount in all leachate samples).
[‡]Sum of pesticide amount retained in the above-ground biobed (Figure 5.12 represents pesticides amounts at 0 to 15, 15 to 30, and 30 to 60-cm depth).
[§]Degradation = 100% - (amount leached + amount retained in biobed mix)%.
[¶]Percentage leached or retained was less than 0.01% of the applied amount.

life of diphenamid was reduced from 25 to 5 d after four successive applications in soil (Avidov et al., 1988).

Temperature is an important factor that affects the loss of pesticides in the soil environment. Increase in temperature could stimulate the growth of microorganisms and higher temperature favours the loss of pesticides through volatilization and non-biological (chemical) degradation (Zhang et al., 1993). In 2010, pesticides were applied in late July to early August due to wet weather conditions and pesticides were monitored from July to October 2010. The average temperature (July to October, 2010) was 12.7 ^{o}C (Environment Canada, 2011) and the average estimated MB-C was 0.4 and 0.3 mg kg^{-1} C in the BGB and AGB, respectively (estimated made in October). In 2011, pesticide application was from June 22 to July 22, 2011. Pesticides were monitored from June to to August 2011. The average temperature (June to August, 2011) was 16.8 ^{o}C (Environment Canada, 2011). This increase in temperature (4.0 ^{o}C) could have had an impact on pesticide degradation as well as the microbial population. Microbial biomass-C estimated on June 17, 2011 was 0.6 mg kg^{-1} C in both biobeds. However, by mid August (2011) the estimated MB-C was 52 times higher from both biobeds compared to the estimate in June. Hence the enhanced degradation of pesticides observed in the second year could have been as a result of higher temperature, the effects of repeated applications and increase in the microbial population.

5.5 Conclusion

This study shows that hydraulic loading and biobed depth are critical for biobed efficiency because high precipitation in 2010 led to leaching of all pesticides from both biobeds. The AGB was more vulnerable to leaching compared to the BGB, and this could have been as a result of its shallower depth and smaller reservoir. The biobed mix used in both biobeds did not degrade metsulfuron-methyl very well. Therefore more research is need by testing various biobed mix ingredients. As a result, caution is advised when placing metsulfuron-methyl and similarly persistent pesticides into a biobed made of the straw, cattle manure and topsoil, based on this study. Based on the 2010 and 2011 results, the AGB is not as suitable as the BGB because: 1) the system was more vulnerable to pesticide leaching, and 2) some of the biobed material was still frozen in

early May which may limit its utility for early season use. However, it could be installed beside the BGB to further treat excessive leachate coming out from the BGB. The BGB could be recommended to farmers to treat spills arising from filling operations (when the sprayer is stationed on the biobed) and wastewater from sprayer cleaning.

6.0 GENERAL DISCUSSION AND CONCLUSIONS

The use of pesticides in agriculture can pose a threat to human health and the environment if inadequately managed (Tortella et al., 2010). Routine monitoring of environmental water has showed the presence of pesticide residues (Fogg et al., 2004b). Point-source contamination due to tank filling or equipment cleaning has been identified as a major source of surface water and groundwater contamination by pesticides (Tortella et al., 2012). Therefore, it is important to develop a system for the containment and treatment of pesticide spills arising from point-source contamination. One such system is the biobed developed in Sweden in 1993 with the aim of reducing point-source contamination by pesticides (Torstensson and Castillo, 1997). Various studies conducted in Sweden (Castillo et al., 2001), the UK (Fogg et al., 2003a), Greece (Karanasios et al., 2010 a,b), Belgium (De Wilde et al., 2010) with biobeds have shown that biobeds can successfully reduce point-source contamination of surface water and groundwater by pesticides. No extensive research has been carried out in Saskatchewan nor Canada to test the efficiency of biobeds under Canadian climatic conditions using locally available materials.

The objectives of this study were to: 1) investigate if net CO_2 production could be used as an indicator for pesticide degradation in a biobed mix and topsoil, 2) study the degradation of seven pesticides in a biobed mix under three temperatures, and 3) compare two field designs of biobed regarding pesticide leaching and degradation, water balance, and temperature. This study is the first of its kind to develop and study biobeds in Saskatchewan. Results from this study will form the basis for any development of biobeds in Saskatchewan or Canada as a whole.

Degradation of pesticides in the soil environment can be measured either by measuring the pesticide concentration (direct method) or indirectly through the parent molecule bioconversion by measuring changes in pH, CO_2 production, and DOC (Govind et al. 1997). Net CO_2 production has been used to study the degradation of polymer materials (Spitzer et al., 1996; Lee et al., 2000). However, this technique had not been exploited in the degradation of pesticides in biobeds. Two studies (Henriksen et al., 2003, Vischetti et al., 2007) linked CO_2 production to MB-C in the biobed but no studies have linked the degradation of pesticides in biobed mix to CO_2 production. Net CO_2

production and chemical analysis were used to determine the degradation of 2,4-D DMA in the biobed mix and topsoil in both single and multiple 2,4-D DMA additions (chapter 3). During 28 d of incubation at 20 ^{o}C, 46.57 and 60.26% C added as a.i. was mineralized in the biobed mix and topsoil, respectively. The mineralization value from topsoil was within the range (50%) required for a substance to be classified as biodegradable (Grady, 1985; Strotmann et al., 2004). Chemical analysis showed that 99.9% of 2,4-D was degraded in the biobed mix after a surge in CO_2 production within 10 d. In the topsoil, no surge in CO_2 production was observed and 35% of the applied amount of 2,4-D DMA was degraded in 28 d.

The MB-C (Figure 3.6) estimate from the biobed mix to which 2,4-D DMA was added (single addition) decreased over time (10 d). However, the impact of 2,4-D DMA on the microbial population, net CO_2 production, and degradation data were inconclusive. Hence, more research is needed in this area. Other studies have showed a reduction in MB-C following pesticide application. A 25 and 60% reduction in MB-C was noticed when chlorpyrifos at a concentration of 10 and 50 mg a.i kg^{-1}, respectively was added to a biobed mix (Vischetti et al., 2007). Furthermore, MB-C was negatively impacted when chlorpyrifos and metalaxyl were applied individually or together to the biobed mix (Vischetti et al., 2008). Similar to the current study with 2,4-D DMA, these studies suggest that 2,4-D DMA, chlorpyrifos or metalaxyl degraders constituted a small portion of the total microbial biomass.

In the multiple 2,4-D DMA additions experiment, 94 and 51% C added as a.i. was mineralized in the biobed mix and topsoil, respectively, after 60 d of incubation at 15 ^{o}C. Chemical analysis showed that 99.7 ± 0.8 and 70 ± 1.4% of the applied amount of 2,4-D DMA was degraded in the biobed mix and topsoil, respectively, within the same period. The enhanced degradation of 2,4-D in topsoil (70%) did not reflect the mineralization data (51.3%). Maybe the added C could have ended-up in the production of new microbial cells or formation of intermediate metabolites that could not be degraded by the present microbial population (Grady, 1985; Calmon et al., 2000). It could be possible that the CO_2 trap (KOH) did not capture all CO_2 produced from topsoil.

The enhanced degradation of 2,4-D in topsoil could have been as a result of 2,4-D DMA multiple additions. Repeated application of the same pesticide to a substrate is

believed to enhance the proliferation of the microbial population that can use the pesticide as a source of C and energy (Arbeli and Fuentes 2007). Torstensson et al. (1975) reported a reduction in 2,4-D half-life in soil from 10 to 4 weeks after repeated applications. Net CO_2 production compared with chemical analyses data in this study shows that it could be used as an indicator for 2,4-D DMA degradation in both the biobed mix and topsoil used in this study.

The effect of temperature on pesticide degradation was studied in the biobed mix (Chapter 4). For all pesticides under investigation, more degradation occurred at 20 ^{o}C followed by that at 13 and 5 ^{o}C, respectively. Castillo et al. (2007) reported similar results. A significant interaction (sampling time and temperature) was observed for thifensulfuron-methyl, pyrasulfotole, 2,4-D DMA, and bromoxynil. While for metsulfuron-methyl and tribenuron-methyl, their degradation was a function of temperature. Calculated activation energy and temperature coefficient (Q_{10}) for tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, and bromoxynil supported the degradation data except for pyrasulfotole. This suggests that the degradation of these pesticides will increase with increase in temperature. For pyrasulfotole, its degradation in the biobed mix could be affected by other factors such as pH, OM, moisture because its degradation is not temperature dependent. Metsulfuron-methyl degraded slowly (< 38% of the applied amount 6 g kg^{-1}) at all temperatures, perhaps as a result of the biobed mix pH (7.4) because at neutral pH, metsulfuron-methyl persist in soil and degrades at a very slow rate (Wang et al., 2008). Smith (1986) reported half-life values of 70, 102, and 178 d at pH 5.2, 6.8, and 7.5, respectively, for metsulfuron-methyl in soil. This shows that biobed mixes with pH (> 5) may not be suitable for metsulfuron-methyl degradation. Results from this experiment show that high degradation of applied pesticides in the biobed will occur during warm conditions (summer) compared to fall or spring.

Two designs of field biobeds (the traditional BGB and the newly developed AGB) were tested during two growing seasons. Moisture management was a major issue for both systems. The amount of leachate pumped out during the entire study showed that the lower part of the biobeds was constantly saturated. In 2010 with more than average rainfall for Saskatoon (653 mm), both biobeds were very vulnerable to pesticide leaching.

The cumulative amount (% applied amount) of each pesticide that leached from beneath the BGB from July 23, 2010 to June 22, 2011 was 27, 1, 2, 1, 1, and 0.04% for metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D DMA, respectively. Meanwhile, 35, 10, 8, 9, 12, and 0.2% of the applied amount of metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D DMA, respectively, leached beneath the AGB. In 2011, with average precipitation for Saskatoon (245-mm) and the BGB covered during most of the pesticide application period, cumulative pesticide loss from June 23 to August 19 was less than 0.5% except for metsulfuron-methyl (13.8%). During the same period of time, 66.3, 5.7, 4.5, 5.7, 7.7, and 0.9% of the applied amount of metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D DMA, respectively, leached from the AGB.

The vulnerability of the AGB to pesticide leaching could have been due to its shallower depth (≈ 75 cm), shallow reservoir, and absence of grass cover in the second year. In a biobed with a depth of 0.5 m, 100, 48.3, and 0.2% of metsulfuron-methyl at a hydraulic loading of 1,175, 688, and 202 L m^{-2}, respectively leached out, while 19.3, 18.4 and less than 0.003% leached out in a biobed with a depth of 1 m under the same hydraulic loading (Fogg et al., 2004a). These results show that for any biobed design, depth and hydraulic loading should be taken into consideration.

The BGB degraded 60.3, 85.8, 88.5, 81.2 79.9 and 99.9% of applied metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, thiencarbazone-methyl, pyrasulfotole, and 2,4-D DMA, respectively. In the AGB with the exception of metsulfuron-methyl (80.1%), more than 96.2% of applied pesticides degraded during two growing seasons.

This study shows that biobeds could be recommended for use in Saskatchewan in particular and the prairie region in general to protect surface water and groundwater contamination by pesticides.

7.0 REFERENCES

Adeboye, M.K.A., A. Bala, A.O. Osunde, A.O. Uzoma, A.J. Odofin, and B.A Lawal. 2011. Assessment of soil quality using soil organic carbon and total nitrogen and microbial properties in tropical agro ecosystems. Agric. Sci. 2:34-40.

Agricultural Research Service, (ARS) 2010. www.ars.usda.gov/Services/docs.htm?docid=14147.

Aktar, M.W., D. Sengupta, and A. Chowdhury. 2009. Impact of pesticide use in agriculture: their benefits and hazards. Interdisc. Toxicol. 2:1-12.

Alexander, M. 1981. Biodegradation of chemicals of environmental concern. Science 211:132-138.

Andersen, S.M., P.B. Hertz, T. Holst, R. Bossi, and C.S. Jacobsen. 2001. Mineralisation studies of ^{14}C-labelled metsulfuron-methyl, tribenuron-methyl, chlorsulfuron and thifensulfuron-methyl in one Danish soil and groundwater sediment profile. Chemosphere 45:775-782.

Anderson, T-H., and K.H. Domsch. 1990. Application of eco-physiological quotients (qCO_2 and qD) on microbial biomasses from soils of different cropping histories. Soil Biol. Biochem. 22:251-255.

Arbeli, Z., and C.L. Fuentes. 2007. Review: Accelerated biodegradation of pesticides: An overview of the phenomenon, its basis and possible solutions; and a discussion on the tropical dimension. Crop Protect. 26:1733-1746.

Arias-Estévez, M., E. López-Periago, E. Martínez-Carballo, J. Simal-Gándara, J.C. Mejuto, and L. García-Río. 2008. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. Agr. Ecosyst. Environ. 123:247-260.

Aust, S.D. 1995. Mechanisms of the degradation by white rot fungi. Environ. Health. Perspect. 103:59-61.

Avidov, E., N. Aharonson, and J. Katan. 1988. Accelerated degradation of diphenamid in soils and means for its control. Weed Sci. 36:519-523.

Ayranci, E., and N. Hoda. 2004. Adsorption of bentazon and propanil from aqueous solutions at the high are activated carbon-cloth. Chemosphere 57:755-762.

Bartha, R., and D. Pramer. 1965. Features of a flask and method for measuring the persistence and biological effects of pesticides. Soil Sci. 100:68-70.

Bartha, R., R.P. Lanzilotta, and D. Pramer. 1967. Stability and effects of some pesticides in soil. App. Microbiol. 1:67-75.

Bauters, T.W.J., T.S. Steenhuis, D.A. DiCarlo, J.L. Nieber, L.W. Dekker, C.J. Ritsema, J.-Y. Parlange, and R. Haverkamp. 2000. Physics of water repellent soils. J. Hydrol. 233-243.

Bending, G.D., and M.S. Rodríguez-Cruz. 2007. Microbial aspects of the interaction between soil depth and biodegradation of the herbicide isoproturon. Chemosphere 66:664-671.

Bending, G.D., D.M. Friloux, and A. Walker. 2002. Degradation of contrasting pesticides by white rot fungi and its relationship with ligninolytic potential. FEMS Microbiol. 212:59-63.

Berglöf, T., W. Koskinen, M.J. Duffy, K.A. Norberg, and H. Kylin. 2003. Metsulfuron-methyl sorption-desorption in field-moist soils. J. Agric. Food Chem. 51:3598-3603.

Bhattacharjeel, A.K., and P. Dureja. 2002. Light-induced transformation of tribenuron-methyl on glass, soil, and plant surface. J. Environ. Sci. Health B37 2:131-140.

Blumhorst, M.R. 1996. Experimental parameters used to study pesticide degradation in soil. Weed Tech. 10:169-173.

Boivin, A., S. Amellal, and M. Schiavon. 2005. 2,4-dichlorophenoxyacetic acid (2,4-D) sorption and degradation dynamics in three agricultural soils. Environ. Pollut. 138:92-99.

Bolan, N.S., and S. Baskaran. 1996. Biodegradation of 2,4-D herbicide as affected by its adsorption-desorption behaviour and microbial activity of soils. Aust. J. Soil Res. 34:1041-53.

Bondarenko, S., J. Gan, D.L. Haver, and J.N. Kabashima. 2004. Persistence of selected organophosphate and carbonate insecticides in waters from a coastal watershed. Environ. Toxicol. Chem. 23:2649-2654.

Boopathy, R. 2000. Factors limiting bioremediation technologies. Bioresour. Technol. 74:63-67.

Bousaba, B., A. Zertal, J. Beguet, N. Rouard, M. Devers, C. Martin, and F. Martin-Laurent. 2009. Evidence for 2,4-D mineralisation in Mediterranean soils: impact of moisture content and temperature. Pest Manag. Sci. 65:1021-1029.

Bromilow, R.H., A.A. Evans, and P.H. Nicholls. 1999. Factors affecting degradation rates of five triazole fungicides in two soil types: 1. Laboratory incubations. Pestic. Sci. 55:1129-1134.

Brown, H.M. 1990. Mode of action, crop selectivity, and soil relations of the sulfonylurea herbicides. Pestic. Sci. 29:263-281.

Brown, H.M., M.M. Joshi, A.T. Van, T.H. Carski, J.J. Dulka, M.C. Patrick, R.W. Reiser, R.S. Livingston, and J. Doughty. 1997. Degradation of thifensulfuron-methyl in soil: role of microbial carboxyesterase activity. J. Agric. Food Chem. 45: 955-961.

Calmon, A., L. Dusserre-Bresson, V. Bellon-Maurel, P. Feuilloley, and F. Silvestre. 2000. An automated test for measuring polymer biodegradation. Chemosphere 41: 645-651.

Cambon, J-P., J. Bastide, and D. Vega. 1998. Mechanism of thifensulfuron-methyl transformation in soil. J. Agric. Food Chem. 46:1210-1216.

Carson, R. 1962. Silent Spring. http://www.scribd.com/doc/17680393/Rachel-Carson-Silent-Spring-19622. (Accessed 25 Jan. 2012).

Carter, A. 2000. How pesticides get into water and proposed reduction measures. Pestic. Outlook 11:149-156.

Carvalho, F.P., and R.J. Hance. 1993. Pesticides in the tropical marine environments: Assessing their fate. IAEA Bull.

Castillo, M.d.P., P. Ander, and J. Stenström. 1997. Lignin and manganese peroxidase activity in extracts from straw solid substrate fermentations. Biotechnol. Tech. 11:701-706.

Castillo, M.d.P., P. Ander, J. Stenström, L. Torstensson, 2000. Degradation of the herbicide bentazon as related to enzyme production by *Phanerochaete*

chrysosporium in two solid substrate fermentation systems. World J. Microbiol. Biotechnol. 16:289-295.

Castillo, M.d.P., A. Anderson, P. Ander, J. Stenström, and L. Torstensson. 2001. Establishment of the white rot fungus *Phanerochaete chrysosporium* on unsterile straw in solid substrate fermentation system intended for degradation of pesticides. World J. Microbiol. Biotechnol. 17:627-633.

Castillo, M.d.P., and L. Torstensson. 2007. Effect of biobed composition, moisture, and temperature on the degradation of pesticides. J. Agric. Food Chem. 55:5725-5733.

Castillo, M.d.P., L. Torstensson, and J. Stenström. 2008**.** Biobeds for environmental protection from pesticides uses - A review. J. Agric. Food Chem. 56:6206-6219.

Cessna, A.J., K.B. Best, R. Grover, and W. Nicholaichuk. 1997. Potential for contamination of the South Saskatchewan River with herbicides applied in irrigation district. Can. Wat. Res. J. 22:237-248.

Cessna, A.J., and J.A. Elliott. 2004. Seasonal variation of herbicide concentrations in prairie farm dugouts. J. Environ. Qual. 33:302-315.

Cessna, A.J., D. B. Donald, J. Bailey, M. Waiser, and J. V. Headley. 2006. Persistence of the sulfonylurea herbicides thifensulfuron-methyl, ethametsulfuron-methyl, and metsulfuron-methyl in farm dugouts (Ponds). J. Environ. Qual. 35:2395-2401.

Cessna, A.J., J.A. Elliott, and J. Bailey. 2010. Leaching of three sulfonylurea herbicides during sprinkler irrigation. J. Environ. Qual. 39:365-274.

Chelme-Ayala, P., M.G. El-Din, and D.W. Smith. 2010. Degradation of bromoxynil and trifluralin in natural water by direct photolysis and UV plus H_2O_2 advanced oxidation process. Wat. Res. 44:2221-2228.

Cheng, W., and R.A. Virginia. 1993. Measurement of microbial biomass in arctic tundra soils using fumigation-extraction and substrate-induced respiration procedures. Soil Biol. Biochem. 25:135-141.

Chowdhury, A., S. Pradhan, M. Saha, and N. Sanyal. 2008. Review: Impact of pesticides on soil microbiological parameters and possible bioremediation strategies. Ind. J. Microbiol. 48:114-127.

Coppola, L., M.d.P. Castillo, E. Monaci, and C. Vischetti. 2007. Adaptation of the biobed composition for chlorpyrifos degradation to Southern Europe conditions. J. Agric. Food Chem. 55:396-401.

Cortassa, S., M.A. Aon, and P. F. Villon. 2001. A method for quantifying rates of O_2 consumption and CO_2 production in soil. Soil Sci. 166:68-77.

Cranmer, B.K., P. Westra, and R.L. Zimdahl. 1999. Sorption, dissipation, and leaching of metsulfuron in Colorado soils. Weed Sci. 47:353-359.

CropLife Canada. 2010. www.croplife.ca/web/english/plant_science_industry (Accessed 27 Feb. 2012).

Crowe, A.S., and W.G. Booty. 1995. A multi-level assessment methodology for determining the potential for groundwater contamination by pesticides. Environ. Monit. Assess. 35:239-261.

Cullimore, D.R. 1981. The enumeration of 2,4-D degraders in Saskatchewan soils. Weed Sci. 29:440-443.

Cunningham, D.S., and R.W. Berti. 1993. Remediation of contaminated soils with green plants: An overview. In vitro Cell. Dev. Biol. 29:207-212.

Dalvie, M.A., E. Cairncross, A. Solomon, and L. London. 2003. Contamination of rural surface and ground water by endosulfan in farming areas of the Western Cape, South Africa. Environ. Health: A Global Access Science Source, 2:1-15.

Damalas, C.A., G.K. Telidis, and S.D. Thanos. 2008. Assessing farmers' practices on disposal of pesticide waste after use. Sci. Total Environ. 390:341-345.

Davis, R.F., A.W. Johnson, and R.D. Wauchope. 1994. Accelerated degradation of phenemiphos and its metabolites in soil previously treated with phenemiphos. J. Nematol. 25:679-685.

De Polli, H., A. Costantini, R. Romaniuk, and M.S.A. Pimentel. 2007. Chloroform fumigation-extraction labile C pool (microbial biomass C “plus”) shows high correlation to microbial biomass C in Argentinian and Brazilian soil. CI Suelo, 25:15-22.

De Roffignac, L., P. Cattan, J. Mailloux, D. Herzog, and F. Bellec. 2008. Efficiency of a bagasse substrate in a biological bed system for the degradation of glyphosate,

malathion and lambda-cyhalothrin under tropical climate conditions. Pest Manag. Sci. 64:130-1313.

De Wilde, T., P. Spanoghe, C. Debaer, J. Ryckeboer, D. Springael, and P. Jaeken. 2007. Review: Overview of on-farm bioremediation systems to reduce the occurrence of point source contamination. Pest Manag. Sci. 63:111-128.

De Wilde, T., P. Spanoghe, K. Sniegowksi, J. Ryckeboer, P. Jaeken, and D. Springael. 2010. Transport and degradation of metalaxyl and isoproturon in biopurification columns inoculated with pesticide-primed material. Chemosphere 78:56-60.

Debear, C., and P. Jaeken. 2006. Modified bio filters to clean-up leftovers from spray loading and cleaning; experience from pilot installations. Asp. App. Biol. 77:247-252.

Degenhardt, D., A.J. Cessna, R. Raina, A. Farenhorst, and D.J. Pennock. 2011. Dissipation of six acid herbicides in water and sediment of two Canadian prairie wetlands. Environ. Toxicol. Chem. 30:1982-1989.

Di, H.J., L.A.G. Aylmore, and R.S. Kookane. 1998. Degradation rates of eight pesticides in surface and subsurface soils under laboratory and field conditions. Soil Sci. 163:404-411.

Digrak, M., and S. Özçelik. 1998. Effect of some pesticides on soil microorganisms. Bull. Environ. Contam. Toxicol. 60:916-922.

Dinelli, G., A. Vicari, A. Bonetti, and P. Catizone. 1997. Hydrolytic dissipation of four sulfonylurea herbicides. J. Agric. Food Chem. 45:1940-1945.

Donald, D.B., A.J. Cessna, E. Sverko, and N.E. Glozier. 2007. Pesticides in surface drinking-water supplies of the Northern Great Plains. Environ. Health Perspect. 115:1183-1191.

Ecobichon, D.J. 2001. Pesticide use in the developing countries. Toxicology 160:27-33.

Edwards, C.A. 1975. Factors that affects the persistence of pesticides in plants and soils. Pure. App. Chem. 43:39.

El-Din Sharabi, N., and R. Bartha. 1993. Testing of some assumptions about biodegradability in soil as measured by carbon dioxide evolution. App. Environ. Microbiol. 59:1201-1205.

Entry, J.A., W.H. Emmingham, and P.K. Donnelly. 1994. Microbial mineralization of atrazine and 2,4-dichlorophenoxyacetic acid in riparian pasture and forest soils. Biol. Fert. Soils 18:89-94.

Entry, J.A., P.K. Donnelly, and W.H. Emmingham. 1995. Atrazine and 2,4-D mineralization in relation to microbial biomass in soils of young-, second-, and old-growth riparian forests. App. Soil Eco. 2:77-84.

Environment Canada. 2011. www.climate.weatheroffice.gc.ca/climateData/dailydata_e.html. (Accessed Dec. 10, 2011).

Environmental Protection Agency (EPA). 2007. Pesticides industry sales and usage. www.epa.gov/opp00001/pestsales/07pestsales/market_estimates2007.pdf (Assessed 10 Jan. 2012).

Environmental Protection Agency (EPA). 2013 www.iaspub.epa.gov/apex/pesticides/f?p=HHBP:home. (Assessed 15 Jan. 2013).

Estrella, M.R., M.L. Brusseau, R.S. Maier, I.L. Pepper, P.J. Wierenga, and R.M. Miller, 1993. Biodegradation, sorption and transportation of 2,4-dichlorophenoxyacetic acid in saturated and unsaturated soils. Appl. Environ. Microbio. 59:4266-4273.

Etinosa, I.O., O. Ajisebutu, A.O. Stella, and O.I. Anthony. 2007. Studies on aerobic biodegradation activities of 2,4-dichlorophenoxyacetic acid by bacteria species isolated from petroleum-polluted site. African J. Biotechnol. 6:1426-1431.

Fahl, G.M., L. Kreft, R. Altenburger, M. Faust, W. Boedeker, and H.L. Grimme. 1995. pH-Dependent sorption, bio concentration and algal toxicity of sulfonylurea herbicides. Aquat. Toxicol. 31:175-187.

Fait, G., M. Nicelli, G. Frangoulis, M. Trevisan, and C. Ettore. 2007. Reduction of point contamination sources of pesticides from a vineyard farm. Environ. Sci. Technol. 41:3302-3308.

Fava, L., M.A. Orrù, A. Crobe, A.B. Caracciolo, P. Bottoni, and E. Funari. 2005. Pesticide metabolites as contaminants of groundwater resources: assessment of the leaching potential of endosulfan sulphate, 2,6-dichlorobenzoic acid, 3,4-dichloroaniline, 2,4-dichlorophenol and 4-chloro-2-methylphenol. Microchem. J. 79:207-211.

Felding, G. 1995. Leaching of phenoxyalkanoic acid herbicides from farmland. Sci. Total Environ. 168:11-18.

Felsot, A.S., K.D. Racke, and D.J. Hamilton. 2003. Disposal and degradation of pesticides waste. Rev. Environ. Cont. Toxicol. 177:123:200.

Flaming, L.E., O. Gómez-Marin, D. Zheng, F. Ma, and D. Lee. 2003. National health interview survey mortality among US farmers and pesticide applicators. Am. J. Ind. Med. 43:227-233.

FOCUS, (2006). Guidance document on estimating persistence and degradation kinetics from environmental fate studies on pesticides in EU registration, report of the FOCUS work group on degradation kinetics, EC document reference Sanco/10058/2005 version 2.0.

Fogg, P., A.B.A. Boxall, A. Walker, and A. Jukes. 2003a. Degradation of pesticides in biobeds: the effect of concentration and pesticide mixtures. J. Agri. Food Chem. 51:5344-5349.

Fogg P., A.B.A. Boxall, A. Walker, and A.A. Jukes. 2003b. Pesticide degradation in a 'biobed' composting substrate. Pest Manag. Sci. 59:527-537.

Fogg, P., A.B.A. Boxall, A. Walker, and A. Jukes. 2004a. Effect of different soil textures on leaching potential and degradation of pesticides in biobeds. J. Agric. Food Chem. 52:5643-5652.

Fogg, P., A.B.A. Boxall, A. Walker, and A. Jukes. 2004b. Leaching of pesticides from biobeds: Effect of biobed depth and water loading. J. Agric. Food Chem. 52:6217-6227.

Fogg, P., A.B.A. Boxall, A. Walker, and A. Jukes. 2004c. Degradation and leaching potential of pesticides in biobed systems. Pest Manag. Sci. 60:645-654.

Fomsgaard, I.S. 1995. Degradation of pesticides in subsurface soils, unsaturated zone-a review of methods and results. Int. J. Environ. Anal. Chem. 58:231-245.

Fournier, J.C. 1980. Enumeration of the soil micro-organisms able to degrade 2,4-D by metabolism or co-metabolism. Chemosphere 9:169-174.

Fox, D.J.C., and P.A. Thompson. 1971. A thermo-gradient apparatus designed for biological investigations over controlled temperature ranges. J. Exp. Bot. 22:741-748.

Gabriela, A., R.I. Vázquez-Rodríguez, C.A Beltrán-Hernández, Lucho-Constantino, and J.L. Blasco. 2007. A method for measuring the anoxic biodegradability under denitrifying conditions. Chemosphere 71:1363-1368.

García-Rodríguez, J., M. García-Martín, M. Nogueras-Ocaña, J. De Dios Luna-del-Castillo, M.E García, N. Olea, and P. Lardelli-Claret. 1996. Exposure to pesticides and cryptorchidism: geographical evidence of a possible association. Environ. Health. Perspect. 104:1090-1095.

Garry, V.F., D. Schreinemachers, M.E. Harkins, and J. Griffith. 1996. Pesticide appliers, biocides, and birth defects in rural Minnesota. Environ. Health. Perspect. 104:394-399.

Gaultier, J., A. Farenhorst, J. Cathcart, and T. Goddard. 2008. Degradation of carboxyl-^{14}C 2,4-D and ring-U-^{14}C 2,4-D in 114 agricultural soils as affected by soil organic carbon content. Soil Biol. Biochem. 40:217-227.

Gaultier, J., A. Farenhorst, S.M. Kim, I. Saiyed, P. Messing, A.J. Cessna, and N.E. Glozier. 2009. Sorption-desorpton of 2,4-dichlorophenoxyacetic acid by wetland sediments. Wetlands 29:837-844.

Gevao, B., K.T. Semple, and K.C. Jones. 2000. Bound pesticide residues in soil: A review. Environ. Pollut. 108:3-14

Ghafoor, A., N.J. Jarvis, T. Thierfelder, and T. Stenström. 2011. Measurements and modeling of pesticide persistence in soil at the catchment scale. Sci. Total Environ. 409:1900-1908.

Golovleva, L.A., R.N. Pertsovas, F. Klrnc, and M. Vokounova. 1988. Decomposition of the herbicide bromoxynil in soil and in bacterial cultures. Folia Microbiol. 33:491-499.

Gonod, L.V., F. Martin-Laurent, and C. Chenu. 2006. 2,4-D impact on bacterial communities, and the activity and genetic potential of 2,4-D degrading communities in soil. FEMS Microbiol. Ecol. 58:529-537.

González-Matute, R., D. Figlas, G. Mockel, and N. Curvetto. 2012. Degradation of metsulfuron-methyl by *Agaricus blazei* murrill spent compost enzyme. Bioremed. J. 16:31-37.

Goss, M.J., D.A.J. Barry, and D.L. Rudolph. 1998. Contamination in Ontario farmstead domestic wells and its association with agriculture. Results from drinking water wells. J. Contam. Hydrol. 32:267-293.

Govind, R., C. Gao, L. Lai, and H.H. Tabak. 1997. Continuous, automated and simultaneous measurement of oxygen uptake and carbon dioxide evolution in biological systems. Wat. Environ. Res. 69:73-80.

Grady, C.P.L. Jr. 1985. Biodegradation: Its measurement and microbiological basis. Biotechnol. Bioeng. 27:660-674.

Grover, R., D.T. Waite, A.J. Cessna, W. Nicholaichuk, D.G. Irvin, L.A. Kerr, and K. Best. 1997. Magnitude and persistence of herbicide residues in farm dugouts and ponds in the Canadian prairies. Environ. Toxicol. Chem. 16:638-643.

Guo, L., W.A. Jury, R.J. Wagenet, and M. Flury. 2000. Dependence of pesticide degradation on sorption: nonequilibrium model and application to soil reactors. J. Contam. Hydrol. 43:45-62.

Gupta, V.K., I. Ali, I. Suhas, and V.K. Saini. 2006. Adsorption of 2,4-D and carbofuran pesticides using fertilizer and steel industry wastes. J. Colloid Interface Sci. 299: 556-563.

Gustafson, D.I. 1989. Groundwater ubiquity score: a simple method for assessing pesticide leachability. Environ. Toxicol. Chem. 8:339-357.

Hamby, D.M. 1996. Site remediation techniques supporting environmental restoration activities-a review. Sci. Total Environ. 191:303-224.

Hanify J.A., P. Metcalf, C.L. Nobbs, and K.J. Worsley. 1981. Aerial spraying of 2,4,5-T and human birth malformations: an epidemiological investigation. Science 212:349-351.

Health Canada. 2010. Thiencarbazone-methyl evaluation report. Publication 100119.

Health Canada. 2011. The regulation of pesticides in Canada. www.hc-sc.gc.ca/cps-spc/pubs/pest/_fact-fiche/reg-pesticide/index-eng.php. (Accessed 14 Dec. 2011).

Helweg, A., B. Henrik, H.P.H. Hansen, M. Rabøølle, A. Sonnenborg, and L. Stenvang. 2002. Pollution at and below sites used for mixing and loading of pesticides. Int. J. Environ. Anal. Chem. 82:583-590.

Henriksen, V.V., A. Helweg, N.H. Spliid, G. Felding, and L. Stenvang. 2003. Capacity of model biobeds to retain and degrade mecoprop and isoproturon. Pest Manag. Sci. 59:1076-1082.

Hildebrandt, A., M. Guillamón, S. Lacorte, R. Tauler, and D. Barceló. 2008. Impact of pesticides used in agriculture and vineyards to surface and groundwater quality North Spain. Wat. Res. 42:3315-3326.

Holtze, M.S., S.B. Sørensen, J. Sørensen, and J. Aamand. 2008. Review: Microbial degradation of the benzonitrile herbicides dichlobenil, bromoxynil and ioxynil in soil and subsurface environments. Insights into degradation pathways, persistent metabolites and involved degrader organisms. Environ. Poll. 154:155-168.

Ismail, B.S., M. Sameni, and M. Halimah. 2011. Kinetics of the microbial degradation of 2,4-D and ^{14}C-labeled paraquat in two types of tropical agricultural soils. World App. Sci. J. 14: 324-333.

Issa, Y., F.A. Sham'a, K. Nijem, E. Bjertness, and P. Kristensen. 2010. Pesticide use and opportunities of exposure among farmers and their families: cross sectional studies 1998-2006 from Hebron governorate, occupied Palestinian territory. Environ. Health 9:63.

Jain, K.V. 1992. Disposing of pesticides in the third world. Environ. Sci. Technol. 26:226-228.

James, T.K., P. Klaffenbach, P.T. Holland, and A. Rahman. 1995. Degradation of primisulfuron-methyl and metsulfuron-methyl in soil. Weed Res. 35:113-120.

Kah, M., S. Beulke, and C.D. Brown. 2007. Factors influencing degradation of pesticides in soil. J. Agric. Chem. 55:4487-4492.

Karanasios, E., N.G. Tsiropoulos, D.G. Karpouzas, and C. Ehaliotis. 2010a. Degradation and adsorption of pesticides in compost-based biomixtures as potential substrates for biobeds in Southern Europe. J. Agric. Food Chem. 58:9147-9156.

Karanasios, E., N.G. Tsiropoulos, D.G. Karpouzas, and U. Menkissoglu-Spiroudi. 2010b. Novel biomixtures based on local Mediterranean lignocellosic material: Evaluation for use in biobed systems. Chemosphere 80:914-921.

Karpouzas, D.G., A. Walker, D.S.H. Drannan, and R.J. Froud-Williams. 2001. The effect of initial concentration of carbofuran on the development and stability of its enhanced biodegradation in top-soil and sub-soil. Pest Manag. Sci. 157:72-81.

Kaune, A., R. Fliege, M. Lenz, and R. Ripperger. 2008. Fate of pyrasulfotole in the environment. Pflanzen. Nach. Bayer 61: 73-86.

Khan, M.J., M.S. Zia, and M. Qasim. 2010. Use of pesticides and their role in environmental pollution. World Acad. Sci. Technol. 72:122-128.

Kolpin, D.W., J.E. Barbash, and R.J. Gilliom. 1998. Occurrence of pesticides in shallow groundwater of the United States: Initial results from the National water-quality assessment program. Environ. Sci. Technol. 32:558-566.

Kravvariti, K., N.G. Tsiropoulos, and D.G. Karpouzas. 2010. Degradation and adsorption of terbuthylazine and chlorpyrifos in biobed biomixtures from composted cotton crop residues. Pest Manag. Sci. 66:1122-1128.

Kreuger, J., and L. Törnqvist. 1998. Multiple regression analysis of pesticide occurrence in streamflow related to pesticide properties and quantities applied. Chemosphere 37:189-207.

Kristensen, G.B., S.R. Sorensen, and J. Aamand. 2001. Mineralization of 2,4-D, mecoprop, isoproturon and terbuthylazine in a chalk aquifer. Pest Manag. Sci. 57:531-537.

Kumar, P., A.S. Halepyati, B.T. Pujari, and B.K. Desai. 2007. Effects of integrated nutrient management on productivity, nutrient uptake and economics of maize (*Zea mays* L.) under rainfed condition. Karnatake J. Agric. Sci. 20:462-465.

Kyriakopoulos, G., and D. Doulia. 2006. Adsorption of pesticides on carbonaceous and polymeric materials from aqueous solutions: A review. Sep. purific. Rev. 35:97-191.

Laroche, A.M., and J. Gallichand. 1995. Analysis of pesticide residues in surface and groundwater of a small watershed. Trans. ASABE. 38:1731-1736.

Lee, J.S., B.L. Daniels, D.T.Eberiel, and R.E. Farrell. 2000. Polymer mineralization in soils: Effects of cold storage on microbial populations and biodegradation potential. J. Polym. Environ. 8:81-89.

Leveau, J.H.J., F. Könlg, H. Füchslin, C. Werlen, and J.R. van der Meer. 1999. Dynamics of multigene expression during catabolic adaptation of Ralstonia eutropha JMP134 (pJP4) to the herbicide 2,4-dichlorophenoxyacetate. Mol. Microbiol. 33:396-406.

Macharia, I., D. Mithöfer, and H. Waibel. 2009. Potential environmental impacts of pesticides use in the vegetable sub-sector in Kenya. Afr. J. Hort. Sci. 2:138-151.

Macur, R.E., J.T. Wheeler, M.D. Burr, and W.P Inskeep. 2007. Impacts of 2,4-D application on soil microbial community structure and on populations associated with 2,4-D degradation. Microbiol. Res. 162, 37-45.

Maltseva, O., C. McGowan, R. Fulthorpe, and P. Oriel. 1996. Degradation of 2,4-dichlorophenoxyacetic acid by haloalkaliphillic bacteria. Microbiology 142:1115-1122.

Marrón-Montiel, E., N. Ruiz-Ordaz, C. Rubio-Granados, C. Juaárez-Ramírez, and C.J. Galíndez-Mayer. 2006. 2,4-D-degrading bacterial consortium isolation, kinetic characterization in batch and continuous culture and application for bioaugmenting an activated sludge microbial community. Process Biochem. 41:1521-1528.

Menne, H.J., and B.M. Berger. 2001. Influence of straw management, nitrogen, fertilization and dosage rates on the dissipation of five sulfonylureas in soil. Weed Res. 41:229-244.

Miller, J.J., N. Foroud, B.D. Hill, and C.W. Lindwall. 1995. Herbicides in surface run-off and groundwater under surface irrigation in southern Alberta. Can. J. Soil Sci. 75:145-148.

Mills, M.S., I.R. Hill, A.C. Newcombe, N.D. Simmons, P.C. Vaughan, and A.A. Verity 2001. Quantification of acetochlor degradation in the unsaturated zone using two noval *in situ* field techniques: Comparisons with laboratory-generated data and implications for groundwater risk assessment. Pest Manag. Sci. 57:351-359.

Mohamed, A., A.A. El Hussein, M.A. El Siddig, and A.G. Osman. 2011. Degradation of oxyfluorfen herbicide by soil microorganism biodegradation of herbicides. Biotechnology 10:274-279.

Müller, K., M. Deurer, T. T. Aslam, and B. Clothier. 2010. Soil carbon management and filtering of organic pesticides. 19th World Congress of Soil Science, Soil Solutions for a Changing World 171, 1-6 August 2010, Brisbane, Australia.

Ogawa, S., P. Baveye, J.-Y. Parlange, and T. Steenhuis. 2002. Preferential flow in the field soils. Forma. 17:31-53.

Ogle, R.E., and G.F. Warren. 1954. Fate and activity of herbicides in soil. Weeds. 3:257-273.

Ogram, A.V., R.E. Jessup, L.T. Ou, and P.S.C. Rao. 1984. Effects of sorption on biological degradation rates of (2,4-Dichlorophenoxy) acetic acid in soil. App. Environ. Microbiol. 49:582-587.

Osman, K., G.H. Ibrahim, A.I. Askar, and A.R.A. Alkhail. 2008. Biodegradation kinetics of dicofol by selected microorganisms. Pestic. Biochem. Physiol. 91:180-185.

Ou, L.T., D.F. Rothwell, W.B. Wheeler, and J.M. Davidson. 1978. The effect of high 2,4-D concentration on degradation and carbon dioxide evolution in soils. J. Environ. Qual. 7:241-246.

Pavel, L.V., and M. Gavrilescu. 2008. Overview of ex-situ decontamination techniques for soil cleanup. Environ. Eng. Manag. J. 7:815-834.

Pigeon, O., C. De Vleeschouwer, F. Cors, B. Weickmans, B. De Ryckel, L. Pussemier, P. Debongnie, and M. Culot. 2005. Development of biofilters to treat the pesticides wastes from spraying applications. Comm. Agric. App. Biol. Sci. 70:1003-1012.

Pingali, P.L., and R.V. Gerpacio. 1997. Towards reducing pesticide use for cereal crops in Asia. CIMMYT Economics Working Paper 97-04. Mexico, D.F.: CIMMYT.

Pizzul, L., M.d.P. Castillo, and J. Stenstrom. 2009. Degradation of glyphosate and other pesticides by ligninolytic enzymes. Biodegradation 20:751-759.

Pons, N., and E. Barriuso. 1998. Fate of metsulfuron-methyl in soils in relation to pedo-climatic conditions. Pestic. Sci. 53:311-323.

Prado, A.G.S., and C. Airoldi. 2001. Microcalorimetry of the degradation of the herbicide 2,4-D via the microbial population on a typical Brazilian red Latosol soil. Thermochimica Acta 371:169-174.

Pussemier, L., C. De Vleeschouwer, and P. Debongnie. 2004. Self-made biofilters for on-farm clean-up of pesticides wastes. Outlook Pest Manag. 2:60-63.

Rao, P.S.C., R.S. Mansell, L.B. Baldwin, and M.F. Laurent. 1983. Pesticides and their behaviour in soil and water. Soil Sci. Fact Sheet.

Rath, A.K., B. Ramakrishnan, S. Kumaraswamy, K. Bharati, P. Singla, and N. Sethunathan. 1998. Effects of pesticides on microbial biomass of flooded soil. Chemosphere 37:661-671.

Raymond, J.W., T.N. Rogers, D.R. Shonnard, and A.A. Kline. 2001. A review of structure-based biodegradation estimation methods. J. Hazard. Mater. 84:189-219.

Rodríguez-Rubio, P., E. Morillo, and C. Maqueda. 2006. Sorption of 2,4-D on natural and organic amended soils of different characteristics, J. Environ. Sci. Health. Part B:41:145-157.

Rosenbrock, P., J.C. Munch, I. Scheunert, and U. Dörfler. 2004. Biodegradation of the herbicide bromoxynil and its plant cell wall bound residues in an agricultural soil. Pest. Biochem. Physiol. 78:49-57.

Saha, S., and G. Kulshrestha. 2008. Hydrolysis kinetics of sulfonylurea herbicide sulfosulfuron. Int. J. Environ. Anal. Chem. 88:891-898.

Sakamoto, K., and Y. Oba. 1994. Effect of fungal to bacterial biomass ratio on the relationship between CO_2 evolution and total soil microbial biomass. Biol. Fertil. Soils 17:39-44.

Salameh, P.R., I. Baldi, P. Brochard, C. Raherison, B.A. Saleh, and R. Salamon. 2003. Respiratory symptoms in children and exposure to pesticides. Eur. Respir. J. 22:507-512.

Sangodoyin, A.Y., and D.W. Smith. 1996. Review of information on pesticide residues in the Canadian environment. The Environmentalist 16:187-196.

Santel, H.J. 2012. Thiencarbazone-methyl (TCM) and Cyprosulfamide (CSA) – a new herbicide and a new safener for use in corn. 25th Ger. Conf. Weed Biol. Weed Cont. March 13-15, 2012, Braunschweig, Germany.

Sarmah, A.K., and J. Sabadie. 2002. Hydrolysis of sulfonylurea herbicides in soils and aqueous solutions: a Review. J. Agric. Food Chem. 50:6253-6265.

Sarmah, A.K., R.S. Kookana, and A.M. Alston. 1998. Fate and behaviour of triasulfuron, metsulfuron-methyl, and chlorsulfuron in the Australian soil environment: a review. Aust. J. Agric. Res. 49:775-90.

Sarmah, A.K., R.S. Kookanana, M.J. Duffy, A.M. Alston, and B.D. Harch. 2000. Hydrolysis of triasulfuron, metsulfuron-methyl, and chlorsulfuron in alkaline soil and aqueous solutions. Pest Manage. Sci. 56:463-471.

Scribner, E.A., A. William, W.A. Battaglin, D.A. Goolsby, and E.M. Thurman. 2000. Changes in herbicide concentrations in Midwestern streams in relation to changes in use, 1989-1998. Sci. Total Environ. 248:255-263.

SCSR, 1978. The soils of the Saskatoon map area, number 73B. SCSR- soil survey staff University of Saskatchewan, Saskatoon Saskatchewan.

Sengupta, D., M.W. Akter, S. Purkait, and M. Ganguly. 2009. Impact of quinalphos on microbial biomass and activities in tropical clay loam soil. J. Environ. Agric. Food Chem. 8:1127-1135.

Shymko, J.L., and A. Farenhorst. 2008. 2,4-D mineralization in unsaturated and near-saturated surface soils of an undulating, cultivated Canadian prairie landscape. J. Environ. Sci. Health Part B. 43:34-43.

Si, Y., S. Wang, J. Zhou, R. Hua, and D. Zhou. 2005. Leaching and degradation of ethametsulfuron-methyl in soil. Chemosphere 60:601-609.

Singh, B.K., A. Walker, J.A.W. Morgan, and D.J. Wright. 2003. Effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium. App. Environ. Microbiol. 69:5198-5206.

Singh, D.K. 2008. Biodegradation and bioremediation of pesticide in soil: concept, method and recent developments. Ind. J. Microbiol. 48:35-40.

Skipper, H.D., A.G. Wollum, R.F. Turco, and D.C. Wolf. 1996. Microbial aspects of environmental fate studies of pesticides. Weed Tech. 10:174-190.

Smith, A.E. 1971. Degradation of bromoxynil in Regina heavy clay. Weed Res. 11:276-282.

Smith, A.E. 1986. Persistence of the herbicides ^{14}C chlorsulfuron and ^{14}C metsulfuron-methyl in Prairie soils under laboratory conditions. Bull. Environ. Contam. Toxicol. 37, 698-704.

Smith, A.E., M.P. Sharma, and A.J. Aubin. 1990. Soil persistence of thiameturon (DPX M6316) and phytotoxicity of the major degradation product. Can. J. Soil Sci.70: 485-491.

Smith, A. E., and K. Mortensen. 1991. Degradation of waste 2,4-D using a soil bacterium in a sprayer tank system. Can. J. Soil Sci. 71:243-246.

Soulas, G. 1993. Evidence for the existence of different physiological groups in the microbial community responsible for 2,4-D mineralization in soil. Soil Biol. Biochem. 25:443-449.

Soulas, G., and B. Lagacherie. 2001. Modelling of microbial degradation of pesticides in soils. Biol. Fert. Soils 33:551-557.

Spanoghe, P., A. Maes, and W. Steurbaut. 2004. Limitation of point source pesticide pollution: Results of bioremediation system. Comm. Agric. App. Biol. Sci. 69:719-732.

Spark, K.M., and R.S. Swift. 2002. Effect of soil composition and dissolved organic matter on pesticide sorption. Sci. Total Environ. 298:147-161.

Spitzer, B., C. Mende, M. Menner, and T. Luck. 1996. Determination of the carbon content of biomass- a prerequisite to estimate the complete biodegradation of polymers. J. Environ. Poly. Deg. 4:157-171.

Spliid, N.H., A. Helweg, and K. Heinrichson. 2006. Leaching and degradation of 21 pesticides in a full-scale model biobed. Chemosphere 65:2223-2232.

Starner, K., K.M. Kuivilla, B. Jennings, and G.E. Moon. 1999. Degradation rates of six pesticides in water from the Sacramento River, California. Water-Resources Investigations Report 99-4018B. U.S. Geological Survey, Sacramento, CA.

Statistics Canada, 1996. Quarterly Estimates of the Population of Canada, the Provinces and the Territories, 11-3, Catalogue no. 91-001, Ottawa.

Strotmann, U., P. Reuschenbach, H. Schwarz, and U. Pagga. 2004. Development and evaluation of an online CO_2 evolution test and a multicomponent biodegradation test system. App. Environ. Microbiol. 70:4621-4628.

Struijs, J., and J. Stoltenramp. 1990. Headspace determination of evolved carbon dioxide in a biodegradability screening test. Ecotoxicol. Environ. Safety 19:204- 211.

Taok, M., N. Cochet, A. Pauss, and O. Schoefs. 2007. Monitoring of microbial activity in soil using biological oxygen demand measurement and indirect impedancemetry. Eur. J. Soil Biol. 43:335-340.

Torstensson, L. 2000. Experience of biobeds in practical use in Sweden. Pestic. Outlook 206-211.

Torstensson L., and M.d.P. Castillo. 1997. Use of biobeds in Sweden to minimize environmental spillages from agricultural spraying equipment. Pest Outlook 24-27.

Torstensson, N.T.L., J. Stark, and B. Göransson. 1975. The effect of repeated applications of 2,4-D and MCPA on their breakdown in soil. Weed Res.15:159-164.

Tortella, G.R., O. Rubilar, M. Cea, C. Wulff, O. Martínez, and M.C. Diez. 2010. Biostimulation of agricultural biobeds with NPK fertilizer on chlorpyrifos degradation to avoid soil and water contamination. J. Soil Sci. Plant Nutr. 10:464-475.

Tortella, G.R., O. Rubilar, M.d.P. Castillo, M. Cea, R. Mella-Herrera, and M.C. Diez. 2012. Chlorpyrifos degradation in a biomixture of biobed at different maturity stages. Chemosphere 88:224-228.

Trabue S.L., D.E. Palmquist, T.M. Lydick, and S.K. Singles. 2006. Effects of soil storage on the microbial community and degradation of metsulfuron-methyl. J. Agric. Food Chem. 54:142-151.

University of Saskatchewan's Kernen Crop Research Farm www.usask.ca/weather/kfarm/data/?C=M;O=D (Accessed 15 Jan. 2010).

Vázquez-Rodriguez, G.A., Beltrán-Hernández, C.A. Lucho-Costantino, and J.L. Blasco. 2008. A method for measuring the anoxic biodegradability under denitrifying conditions. Chemosphere 71:1363-1368.

Vidali, M. 2001. Bioremediation. An overview. Pure Appl. Chem. 73:1163-1172.

Villaverde, J., M. Kah, and C.D. Brown. 2008. Adsorption and degradation of four acidic herbicides in soils from southern Spain. Pest Manag. Sci. 64:703-710.

Vischetti, C., E. Capri, M. Travian, C. Casucci, and P. Perucci. 2004. Biomassbed: a biological system to reduce pesticide point contamination at farm level. Chemosphere 55:823-828.

Vischetti, C., L. Coppola, E. Monaci, A. Cardinali, and M.d.P. Castillo. 2007. Microbial impact of the pesticide chlorpyyrifos on Swedish and Italian biobeds. Agron. Sustain. Dev. 267-272.

Vischetti, C., E. Monaci, A. Cardinali, C. Casucci, and P. Perucci. 2008. The effect of initial concentration, co-application and repeated applications on pesticide degradation in a biobed mixture. Chemosphere 72:1739-1743.

Von Wirén-Lehr, S., M.d.P. Castillo, L. Torstensson, and I. Scheunert. 2001. Degradation of isoproturon in biobeds. Biol. Fertil. Soil 33:535-540.

Voos, G., and P.M. Groffman. 1997. Dissipation of 2,4-D and dicamba in a heterogeneous landscape. App. Soil Eco. 5:181-187.

Voroney, R.P., P.C. Brookes, and R.P. Beyaert. 2007. Soil microbial biomass C, N, P, and S. p. 637-651. In M.R. Carter and E.G. Gregorich (2nd ed.) Soil sampling and methods of analysis. 2nd ed. CRC Press, Boca Raton, FL.

Waite, D.T., A.J. Cessna, R. Grover, L.A. Kerr, and A.D. Snihura. 2002. Environmental concentration of agricultural herbicides: 2,4-D and triallate. J. Environ. Qual. 31:129-144.

Waite, D.T., P. Bailey, J.F. Sproull, D.V. Quiring, D.F. Chau, J. Bailey, and A.J. Cessna. 2005. Atmospheric concentration and dry and wet deposits of some herbicides currently used on the Canadian prairies. Chemosphere 58:693-703.

Waite, D.T., R. Grover, N.D. Westcott, H. Sommerstad, and L. Kerr. 1992. Pesticides in groundwater, surface water and spring runoff in a small Saskatchewan watershed. Environ. Toxicol. Chem. 11: 741-748.

Walker, A., and M. Jurado-Exposito. 1998. Adsorption of isoproturon, diuron and metsulfuron-methyl in two soils at high soil:solution ratios. Weed Res. 38:229-238.

Walker, A., M. Jurado-Exposito, G.D. Bending, and V.J.R. Smith. 2000. Spatial variability in the degradation rate of isoproturon in soil. Environ. Poll. 111:407-515.

Wang, H., J. Wu, S. Yates, and J. Gan. 2008. Residues of ^{14}C-metsulfuron-methyl in Chinese paddy soils. Pest Manag. Sci. 1526-498X.

Wang, H., J. Xu, S.R. Yates, J. Zhang, J. Gan, J. Ma, J. Wu, and R. Xuan. 2010. Mineralization of metsulfuron-methyl in Chinese paddy soils. Chemosphere 78: 335-341.

Wang, N.X., Q. Tang, G.M. Ai, Y.N. Wang, B.J. Wang, Z.P. Zhao, and S.J. Liu. 2011. Biodegradation of tribenuron-methyl that is mediated by microbial acidohydrolysis at cell-soil interface. Chemosphere 86:1098-1105.

Wardle, D.A., and D. Parkinson. 1990. Effects of three herbicides on soil microbial biomass and activity. Plant Soil 122:21-28.

Warren, G.T. 1998. Spectacular increase in crop yields in the United States in the twentieth century. Weed Technol. 12:752-760.

Wauchope, R.D. 1978. The pesticide content of surface water draining from agricultural fields - A review. J. Environ. Qual. 7:459-472.

WenHai, C., G. NaiYun, L. Chong, and C. Jing. 2009. Photochemical degradation of typical halogenated herbicide 2,4-D in drinking water with UV/H_2O_2/micro-aeration. Sci. China Ser B-Chem. 52:2351-2357.

Werf van der, H.M.G. 1996. Assessing the impact of pesticides on the environment. Agric. Ecosyst. Environ. 60:81-96.

Williams, D.D., N.E. Williams, and Y. Cao. 1999. Road salt contamination of groundwater in a major metropolitan and development of a biological index to monitor its impact. Water Res. 34:127-138.

Wilson, R.D., J. Geronimo, and J.A. Armbruster. 1997. 2,4-D dissipation in field soils after application of 2,4-D dimethylamine salt and 2,4-D 2-ethylhexyl ester. Environ. Tox. Chem.1239-1246.

Ye, Q., J. Sun, and J. Wu. 2003. Causes of phytotoxicity of metsulfuron-methyl bound residues in soil. Environ. Pollu. 126:417-423.

Zhang S.M., D.S. Liu, Z.S. Wang, and X.F. Ma. 1993. A kinetic model describing the effect of temperature on the loss rate of pesticides in soil. Ecol. Mod. 70:115-125.

Zhu, G., H. Wu, J. Guo, and F.M.E. Kimaro. 2004. Microbial degradation of fipronil in clay loam soil. Wat. Air. Soil Pollut. 153:35-44.

APPENDIX A: TEMPERATURE CALCULATIONS

Pesticide application in Saskatchewan starts in early May. To predict the biobed temperature (Table A1) during this period of the year, average daily soil temperature at a depth of 5-100 cm was calculated from May 1 to 15, for the past 10 years (1999 to 2008). The calculated average daily soil temperature is 6.7 ^{o}C but due to difference in texture between the soil and biobed mix, the start temperature for the laboratory experiment was 5 ^{o}C. The following temperatures (13 and 20 ^{o}C) were also used. These temperatures were arrived at after calculating the daily monthly soil temperature from 0 to 100 cm for the years 2008, 2007, and 2005 (Table A2, Figure A1). These temperatures (5, 13, and 20 ^{o}C) fall within the range of temperatures a typical Saskatchewan biobed is expected to experience during a growing season.

Table A1. Mean daily soil temperatures at various depth from May 1 to 15 for 2008 to 1999 used to obtain 5 ^{o}C as the starting temperature for laboratory experiment

Depths (cm)	2008	2007	2006	2005	2004	2003	2002	2001	2000	1999
5	9.1	10.1	10.0	8.8	7.6	9.6	6.8	12.1	10.5	9.2
10	8.2	9.2	9.6	8.1	73	9.2	6.2	11.2	10.2	8.8
20	6.9	7.5	8.6	7.3	6.8	8.3	5.3	10.1	9.4	8.1
50	3.2	3.2	6.1	5.2	5.4	6.1	3.0	6.3	6.8	5.6
100	0.1	0.1	3.3	2.7	3.6	3.3	1.4	2.9	3.8	2.5
Average	5.5	6.0	7.5	6.4	6.1	7.3	4.5	8.5	8.2	6.8

Table A2. Mean monthly soil temperature at various depths for 2008, 2007 and 2005 used to obtain 13 and 20 ^{o}C as temperatures for the laboratory experiment

						2008						
Depth (cm)	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
5	-5.5	-8.5	-3.4	2.3	11.6	17.0	20.5	20.2	12.9	6.0	0.0	-8.4
10	-5.1	-8.0	-3.5	1.7	10.8	16.2	20.1	19.9	13.2	6.5	0.6	-7.6
20	-4.4	-7.3	-3.6	0.8	9.4	15.0	18.9	19.3	13.4	7.1	1.5	-6.7
50	-2.2	-5.0	-3.3	-0.7	5.6	11.8	15.9	17.3	13.7	9.0	3.9	-2.8
100	0.3	-1.6	-2.3	-1.1	2.1	8.2	12.1	14.4	13.2	10.4	6.3	1.6
Average	-3.4	-6.1	-3.2	0.6	7.9	13.7	17.5	18.2	13.3	7.8	2.4	-4.8
						2007						
5	-4.0	-60	-2.4	3.1	10.6	17.0	23.5	18.3	12.9	6.3	-0.3	-4.8
10	-3.5	-5.5	-2.4	2.5	9.9	16.3	22.9	18.3	13.1	6.7	0.3	-4.1
20	-2.9	-4.9	-2.5	1.2	8.5	14.9	21.4	18.1	13.3	7.3	1.3	-3.2
50	-1.0	-3.0	-2.2	-0.6	5.0	11.7	17.6	17.3	13.7	8.9	3.8	-0.4
100	1.1	-0.2	-1.1	-0.6	2.0	8.1	13.2	15.2	13.3	10.0	6.2	2.5
Average	-2.1	-3.9	-2.1	1.2	7.2	13.6	19.7	17.5	13.3	7.8	2.3	-2.0
						2005						
5	-10.4	-6.4	-4.0	5.7	11.0	16.7	20.9	18.1	12.9	6.3	0.4	-3.2
10	-9.9	-6.3	-3.9	5.0	10.3	16.3	20.5	18.0	13.1	6.6	1.0	-2.7
20	-9.3	-6.0	-4.0	3.9	9.3	15.1	19.4	17.6	13.3	7.2	1.8	-2.0
50	-6.5	-5.0	-3.6	1.1	6.8	12.0	16.7	16.4	13.6	8.6	4.0	0.2
100	-2.1	-3.0	-2.5	-0.8	4.0	8.3	13.0	14.3	13.1	9.8	6.2	2.7
Average	-7.6	-5.4	-3.6	3.0	8.3	13.7	18.1	16.9	13.1	7.7	2.7	-1.0
Total	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
average	**-4.4**	**-5.1**	**-3.0**	**1.6**	**7.8**	**13.6**	**18.4**	**17.5**	**13.2**	**7.8**	**2.5**	**-2.6**

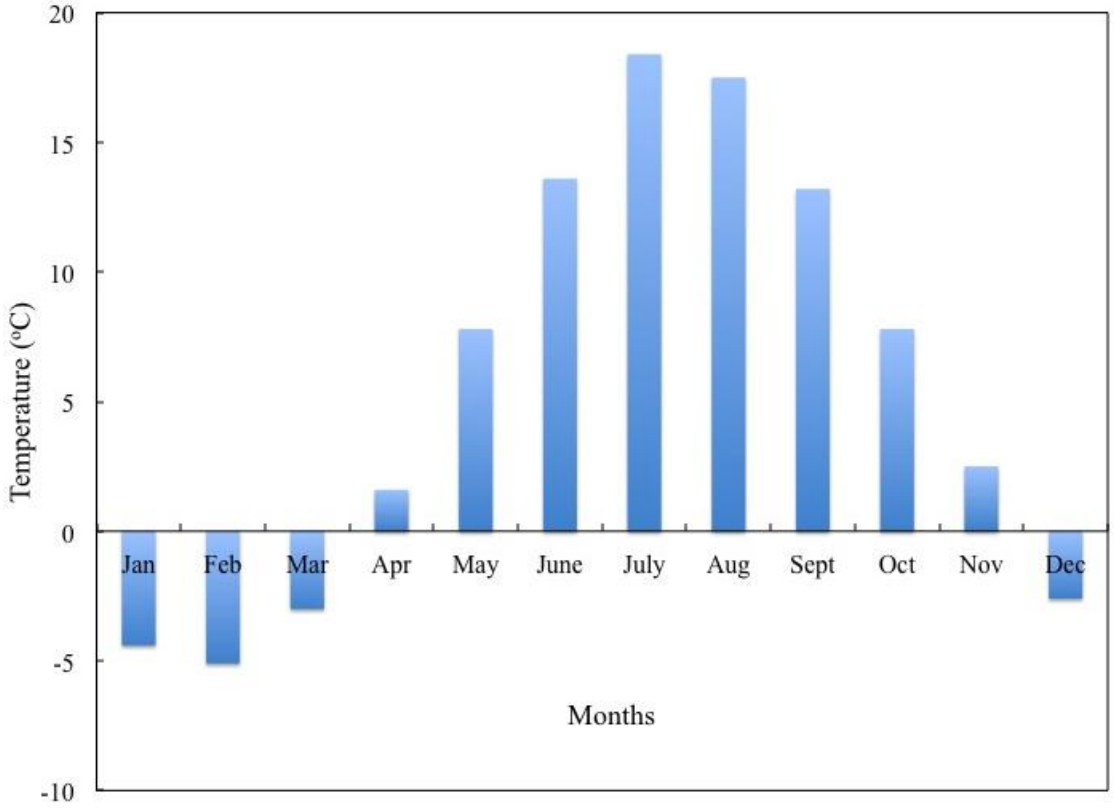

Figure A1 Monthly average soil temperatures for Saskatoon, SK (2008, 2007 and 2005)